HUMAN PROTEIN REQUIREMENTS AND THEIR FULFILMENT IN PRACTICE

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HUMAN PROTEIN REQUIREMENTS AND THEIR FULFILMENT IN PRACTICE

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FOREWORD

THE Princeton Conference on protein requirements was the second conference on proteins in nutrition sponsored jointly by FAO, WHO and the Josiah Macy Jr. Foundation. It was a further step in the development of the programme of FAO, WHO and UNICEF which aims at preventing protein malnutrition, now responsible for much disease and mortality throughout the world. This programme was initiated in 1949, when the joint FAO/WHO Expert Committee on Nutrition, at its First Session, recommended that WHO should collect information about the kwashiorkor syndrome in its epidemiological, nutritional, social and other aspects. In response to this recommendation, FAO and WHO together made surveys of the problem in a number of countries and regions.

At subsequent sessions the Joint Committee reviewed the FAO/WHO activities in this field and proposed means of extending and strengthening them. Its Third Session, in the Gambia in West Africa in 1953, was concerned exclusively with protein malnutrition. Apart from the Joint Committee, regional FAO/WHO nutrition conferences and committees in South-east Asia and Latin America have done a good deal to create interest in the problem and stimulate further enquiries.

In 1953 a number of investigations had been made or were proceeding in different countries, covering the epidemiology, clinical features, biochemistry, pathology, treatment and prevention of protein malnutrition. The results of these showed a basic similarity, but on the other hand there were differences that required elucidation. These might be due to geographical variation in the nature of the syndrome, related to the type of diet associated with it; they might also arise from lack of uniformity on the part of the investigators in their approach to the problem and the interpretation of findings.

In this situation it was clearly desirable to arrange technical and scientific discussions between interested workers in different parts of the world. The first meeting sponsored by FAO, WHO and the Macy Foundation, which took place in Jamaica in 1953, was arranged with this object in view. The subject of this Conference was protein malnutrition in its biochemical, pathological and clinical aspects. Epidemiology and prevention were also discussed, but less exhaustively.

Step by step, as a result of this Conference and contacts and consultations between workers in different countries, a uniform picture emerged. It became clear that the manifestations of protein malnutrition are essentially similar everywhere, even though some local variations still require explanation. Accordingly, at the Princeton Conference, the emphasis was laid on practical measures for dealing with a problem now fairly well understood. The long-term solution depends on making sufficient protein available to the age groups in which protein malnutrition is most likely to occur; this involves in turn appropriate developments in food production and distribution, which must be planned on a sound basis. For this reason, knowledge of protein requirements was one of the central themes of the Princeton Conference. Its review of the subject, even though it revealed large gaps in existing knowledge and the difficulty of defining requirements in a satisfactory way, served a useful purpose. A small FAO Committee on Protein Requirements, which met in Rome in October 1955, made use of this review in attempting to reach more precise conclusions.

In the second stage of its discussions, the Conference dealt with the fulfilment of needs for protein. The additional protein needed to prevent protein malnutrition must come from a variety of animal and vegetable sources, and the wider use of various processed protein-rich preparations should contribute to prevention. Further investigations are, however, needed to develop preparations suitable for young children and careful preliminary testing is essential before their use can be advocated on a large scale. There was unanimous agreement as to how such trials should be conducted.

Since the Princeton Conference, further interest has developed in this field of work, which is of great importance in preventive medicine. The Rockefeller Foundation has made available to the Food and Nutrition Board, National Research Council, U.S.A., a generous grant for research on protein-rich foods and the Committee which will administer this grant will work in collaboration with WHO, FAO and UNICEF. UNICEF has also made financial provision for assisting in testing programmes. Both the Jamaica and Princeton Conferences have contributed to these and other promising developments. They brought together workers from different countries with a common interest in the problem and helped to clarify some controversial and troublesome questions. They also helped to enlist the co-operation of people who, although they may not be directly concerned with protein malnutrition, have knowledge and experience which can contribute to its prevention.

On behalf of FAO and WHO we wish to express gratitude to the Josiah Macy Jr. Foundation and to Dr. Fremont-Smith, its Medical Director, for co-operation in the Conference. The presence at the Conference of Dr. Fremont-Smith, with his experience in guiding discussions and skill in resolving philosophical tangles, was an asset of genuine value. We must also acknowledge gratefully the help of other members of the staff of the Josiah Macy Jr. Foundation who were responsible for the administrative arrangements.

Further thanks are due to Dr. C. G. King, Chairman of the Conference and to Dr. J. C. Waterlow and Dr. J. M. L. Stephen, who undertook the heavy task of editing the report.

W. R. Aykroyd,
Director,
Nutrition Division,
Food and Agriculture Organization
of the United Nations.

R. C. Burgess,
Chief,
Nutrition Section,
World Health Organization.

INTRODUCTION

THE rapid increase in the world population in the last few generations has led to the fear that the human race will outstrip its food supply. The amount of protein available and the way in which it is used are therefore matters of importance to everyone. A number of official committees and scientific organizations in Europe, North America and elsewhere have tried to decide how much protein a human being needs for health and full activity, but usually this has been done against a background of liberal sources and supplies of food; the main impact of the problem, however, is in under-developed countries, where such conditions do not apply. The Princeton Conference provided an opportunity for relating the different points of view and fields of experience.

Since the early committees had only limited evidence before them, they were careful to allow a generous margin of safety in their recommendations. For the individual this is clearly desirable, but if there is a deficit of protein in the world as a whole it is unrealistic and wasteful to set too high a level. The aim must therefore be to strike a balance between economy and safety; if this involves a reduction in the recommended allowances, the evidence on which the decision is taken must be unassailably sound. The Conference showed that for a more exact definition of protein requirements we need, first, to know more about what people eat who are living at a subsistence level, and secondly, better criteria by which to judge whether or not a given intake is adequate. The one depends on accurate observation in the field, the other on work in the laboratory. Evidence of both kinds is presented in this book, but it will easily be seen that the information is by no means complete.

As with most practical problems, there is undoubtedly no single or immediate solution. The world cannot wait for full scientific certainty, and so the end must be approached by successive approximations. It was decided that after the Princeton Conference a smaller group should meet in Rome to formulate conclusions, drawing on the evidence brought out in the discussions at Princeton; their report, shortly to be published, will contain definite recommendations on protein requirements. This plan had the advantage that at Princeton the field was free for arguments to be thrashed out without the restriction of any fixed agenda, a method of scientific communication first introduced by Dr. Fremont-Smith and now generally adopted at conferences of the Macy type. Although for the participants the free discussion and uninhibited interruption is stimulating if at times painful, it does not necessarily follow that the stimulus can be transmitted in a book; the printed page is a poor substitute for a performance in the flesh. This book, like the report of the Jamaica Conference which preceded it, is far from being a verbatim transcript; the original has been ruthlessly pruned and great liberties have been taken with the order and the wording, subject to the guiding principle that the sense must not be altered. Our aim has been not only to preserve, but to bring out the spirit of the original discussion; to produce not a touched-up photograph but a portrait. In addition to its purpose as a record of the proceedings, the book serves to collect together the information which has accumulated in recent years on this important but ill-defined subject. In so doing it makes only too clear the gaps in the evidence, the conflicts and cross-currents of opinion, and the problems which remain to be solved. The result cannot but be rambling and discursive, since all discussions are like that. We hope, however, that these disadvantages may be outweighed

by the interest to be derived from the interplay of different personalities and different disciplines.

The whole Conference, as the title implies, centred round man, and in particular round the child. From this central point the discussions ranged over a wide field, from metabolic experiments on animals to the testing of new foods. It was inevitable that the ground should be covered sketchily, but at the same time in this diversity there was an element of strength. That practical advances depend on research is a truism; but it also happens—and medicine in particular can show many examples—that fundamental research derives its stimulus and its driving force from the desire to satisfy human needs. When the Conference closed, the participants, whether concerned with application or with theory, for the most part acknowledged some benefit from this process of cross-fertilization.

In the summaries attached to each section we have tried to gather together the main conclusions and ideas. It would be impossible to make a factual précis that did justice to every point and every argument; we have therefore chosen what seemed most significant, and for this choice—for errors both of commission and omission—only the editors are responsible.

We should like to record our gratitude to those who attended the Conference for their co-operation, and particularly to Professor Paul Gyorgy for his constant encouragement and interest throughout what proved to be a difficult task; to Miss Patricia Hall, whose devoted work brought the difficulties under control; and to the staff of the Josiah Macy Jr. Foundation, who prepared the original transcript and the index.

J. C. Waterlow
J. M. L. Stephen

LIST OF PARTICIPANTS

DR. J. B. ALLISON, Director of Biological Research, Rutgers University, New Brunswick, New Jersey, U.S.A.

DR. M. AUTRET, Nutrition Division, Food and Agriculture Organization of the United Nations, Rome.

DR. W. R. AYKROYD, Director, Nutrition Division, Food and Agriculture Organization of the United Nations, Rome.

DR. JOSE MARIA BENGOA, Inter-regional Adviser in Nutrition, World Health Organization, Geneva.

DR. B. BORCIC, United Nations International Children's Emergency Fund, United Nations Building, New York, U.S.A.

DR. R. C. BURGESS, Chief, Nutrition Section, World Health Organization, Geneva.

DR. DAMON V. CATRON, Professor of Animal Husbandry, Iowa State College, Ames, Iowa.

DR. E. K. CRUICKSHANK, Professor of Medicine, University College of the West Indies, Jamaica.

DR. WILLIAM J. DARBY, Professor of Biochemistry and Nutrition, Vanderbilt University, Nashville, Tennessee, U.S.A.

DR. R. F. A. DEAN, Medical Research Council, Director, Group for Research in Infantile Malnutrition, Mulago Hospital, Kampala, Uganda, East Africa.

DR. E. DE MAEYER, Institut pour la Recherche Scientifique en Afrique Centrale, Lwiro (Katana), D.S. Bukavu, Belgian Congo.

DR. C. A. ELVEHJEM, Chairman, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, U.S.A.

DR. FRANK FREMONT-SMITH, Medical Director, Josiah Macy Jr. Foundation, New York, U.S.A.

DR. C. GOPALAN, Deputy Director, Nutrition Research Laboratories, Indian Council of Medical Research, Coonoor, South India.

DR. PAUL GYORGY, Professor of Paediatrics, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.

DR. JOHN HANSEN, Department of Paediatrics, University of Cape Town, Union of South Africa.

DR. L. EMMETT HOLT, JR., Professor of Paediatrics, College of Medicine, New York University, New York, U.S.A.

DR. C. G. KING, Scientific Director, Nutrition Foundation, New York, U.S.A.

DR. JEAN MAYER, Assistant Professor of Nutrition, School of Public Health, Harvard University, Boston, Massachusetts, U.S.A.

DR. L. A. MAYNARD, Chief, Division of Biology and Agriculture, National Research Council, U.S.A.

DR. B. S. PLATT, Medical Research Council, Director, Human Nutrition Research Unit and Applied Nutrition Unit, Department of Human Nutrition, London School of Hygiene.

DR. W. C. ROSE, Professor of Chemistry, University of Illinois, Urbana, Illinois, U.S.A.

DR. N. S. SCRIMSHAW, Director, Institute for Nutrition for Central America and Panama, Guatemala City, Guatemala.

DR. W. H. SEBRELL, Director, National Institutes of Health, Bethesda, Maryland, U.S.A.

Dr. J. Sénécal, Professor of Paediatrics, University of Dakar, Dakar, French West Africa.

Dr. F. J. Stare, Professor of Nutrition, School of Public Health, Harvard University, Boston, Massachusetts, U.S.A.

Dr. E. F. Terroine, Director, Centre National de Coordination des Etudes et Recherches sur la Nutrition et l'Alimentation, Paris, France.

Dr. L. Verhoestraete, Adviser in Maternal and Child Health, World Health Organization, Regional Office, Washington, D.C., U.S.A.

Dr. John Waterlow, Medical Research Council, Director, Tropical Metabolism Research Unit, University College of the West Indies, Jamaica.

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I: CONTRIBUTIONS FROM EXPERIMENTAL WORK ON MAN AND ANIMALS

Introduction

OUTLINE OF THE PROBLEM

AYKROYD: I feel diffident in opening this discussion because so many people here have been occupied in this particular field for many years. The original intention was that the late Sir Edward Mellanby would have been the opening speaker, since he was the chairman of the Technical Commission on Nutrition of the League of Nations, which met in 1935, the last international group to consider protein requirements. My statement, for what it is worth, is intended to provide a framework for subsequent discussions. It will certainly raise more questions than it answers. I hope to be able to pass on to others some of the more difficult points which will be brought forward by interrupters.

Normally, a review of protein requirements would begin by an examination of scientific foundations, that is with such questions as the composition of proteins, specific endogenous nitrogen expenditure, the concept of biological value, etc. This is the approach adopted by Professor Terroine in his excellent report.[1] But I am proposing to start from the other end, to refer first to some of the practical implications of the problem and to some of the attempts which have been made to define protein requirements. I hope this may provide a setting for our subsequent discussions, so that when we come to deal with scientific detail the broader practical issues will be kept continually in mind. At Macy conferences one is entitled to stress to some degree one's own preoccupations, and therefore I propose to refer to FAO's responsibilities and particularly to my own personal responsibilities as Director of the Nutrition Division of FAO.

FAO was created to ensure that people get enough of the right sort of food to eat and the word "right" implies the fulfilment of qualitative requirements. It is evident at FAO conferences that governments are becoming increasingly aware of the importance of nutrition and its influence on health and working capacity, and attempts are now being made by governments—not all governments, but some—to plan food production in such a way that the requirements of their peoples are met. This immediately raises such questions as the following: what emphasis should be placed on the development of dairy and fisheries industries? Should processed milk be imported for the consumption of children and mothers?

An example of a practical question related to the subject we are considering arises from a recent recommendation made to UNICEF that the amount of dried skimmed milk to be given to children of different ages should be increased from the usual UNICEF figure of 30–40 g. a day to 60 g. or more. This would involve a much greater expenditure on the distribution of skimmed milk. Therefore I think it would be legitimate for the taxpayers of the world, who provide the UNICEF funds, to ask: "Are there adequate scientific data to justify this increased expenditure of public money?"

We are sometimes asked quite simple and direct questions, such as. "Is milk a necessary food for children?" In existing circumstances, answering such questions in a fully rational and scientific way is by no means easy. Other parallel problems crop up in the course of FAO's work; for example, that of agricultural policy on the cultivation of a food such as manioc as opposed to a cereal, having regard to the difference in protein content of the two kinds of foods.

The problem of protein requirements is of importance not only to FAO but also to WHO in relation to their maternity and child welfare work, and, apart from these international organizations, to many people throughout the world who are concerned with public health and agricultural policy. There is therefore a great need for satisfactory knowledge of protein requirements, and for satisfactory definitions based on that knowledge. Given that need let me briefly refer to some of the attempts that have been made to meet it.

EXISTING DEFINITIONS OF PROTEIN REQUIREMENTS

Aykroyd (continued): I don't think we need go too far back into history. I believe that in about 1860 a certain Dr. E. Smith set up a dietary standard in terms of carbon and nitrogen to avert starvation among cotton workers in Lancashire. We can salute him and pass on. We need not linger either on Carl Voit and his recommendations on protein requirements, which were over-influenced by what he observed among the population of Munich in 1880. As we all know, there have been many attempts by both national and international bodies to establish tables or schedules which purport to show, *inter alia*, the protein requirements of human beings according to age and sex. I have already mentioned one of these bodies—the League of Nations' Technical Commission on Nutrition. In the United States the National Research Council and in the United Kingdom the British Medical Association have also made such attempts, and individual workers and experts in quite a large number of countries—Australia, Canada, India, France, South Africa, etc.—have tried to produce tables which include figures of one kind or another representing protein requirements.

I now propose to examine some of the figures for protein requirements put forward in these tables for the age group 1 to 5, the group in which protein malnutrition occurs most frequently, and to consider the implications of these recommendations with reference to children in the under-developed countries generally—that is, the countries in which protein malnutrition is common.

The League of Nations Committee recommended 3·5 and 3·0 g. of protein per kg. of body weight for the age groups 1 to 3 and 3 to 5 respectively. The various figures for "optimal" intakes given in Mayer's table[2] (see Appendix I) are of the same order, though in general somewhat higher. According to the N.R.C. tables,[3] a child aged 2, weighing 12 kg., should receive 1,200 calories and 40 g. of protein daily, or 3·3 g. per kg. of body weight. Such a body weight would be on the high side for a child of 2 in most parts of the world. For this reason and for purposes of simplicity, let us take a figure of 10 kg. for body weight, a daily calorie requirement of 1,000, and a hypothetical protein requirement of 3·0 g. per kg.

Now we know that in the under-developed countries children on weaning are usually given the ordinary diet of the family. We also know from many sources that in typical family diets some 60 to 70 per cent of total calories is

derived from cereals and starchy roots. Suppose a child of 2–3 years needing 1,000 calories obtains 60 per cent of these from rice: this means that he eats about 165 g. of rice daily from which he gets about 10 g. of protein, or 1·0 g. per kg. body weight. To reach the recommended figure of 3·0 g. per kg. he requires 20 more g. of protein a day. In the form of milk this means about 600 ml. daily, which is usually beyond the bounds of practical possibility; in the form of beans and other pulses it means about 100 g.—a quantity too large for any child of that age to swallow.

You may regard this as a gross over-simplification. Of course the situation will differ according to the staple food of the area. If this is wheat, less protein will be needed from non-cereal sources; if, on the other hand, it is cassava or plantain, then the amount of protein needed from other foods to reach an intake of 3·0 g. per kg. daily will be greatly increased. Again, there will of course be other foods in the diet—for example, some vegetables and perhaps a little meat or fish now and again. But by and large the figures I have given seem to present a rough picture of the situation.

GYORGY: Your argument is based on the premise that 3 g. per kg. is the requirement. If that is not true, then the whole calculation fails. I would like to go on record as saying that I don't know whether the premise is correct.

PLATT: Are we considering the weight of the child as it is or as it should be?

GOPALAN: That is an important point. If diminished body weight is itself the result of diminished protein intake, it would be setting up a vicious circle to base the protein requirement on the actual body weight. This point is particularly important in undernourished communities.

SCRIMSHAW: I agree. We have to determine the protein requirement for the actual weight of the child plus that for optimal growth.

WATERLOW: Are you sure that you need more protein for growth? As Professor Terroine has pointed out,[1] very little extra protein is actually needed for building up body tissue even at times of most rapid growth.

TERROINE: I have been struck by the fact that the figures mentioned so far seem to be purely empirical. What, in fact, is their factual basis? I think it would be more reasonable—and for a physiologist like myself it is essential—to give an exact scientific basis for the figures that are put forward. If one considers the amounts of protein that are laid down in the human body in the course of growth, one finds that they are in fact very small even during the most active periods of growth. Therefore if proteins of high biological value are being used, the needs will be minimal.

I am most anxious that our discussions should lead to clear-cut definitions. Many of our colleagues, and particularly Dr. Autret, have stated that in many cases of malnutrition there is an insufficiency of calories as well as of nitrogen. If many factors vary at the same time it then becomes impossible to know which is responsible for arrest or retardation of growth. What we need is some exact data for man which show the level of protein intake below which growth ceases to be normal, when the diet is in other respects completely adequate.

AYKROYD: The point which I particularly want to make is that most existing recommendations with regard to the protein needs of young children are of little practical help to the people concerned with the problem of protein malnutrition in under-developed countries. Those who put them forward adopted certain underlying assumptions, even though these are not stated in so many words. The background to the tables is a type of diet containing a

considerable variety of foods of both plant and animal origin. The recommendations for children from the age of 1 year onwards assume that animal milk will form a large part of the diet. With respect to most age groups, it is taken for granted that a considerable proportion of protein intake will be in the form of protein of animal origin. The tables are in fact a product of western civilization. Like Voit's standards, though perhaps not so obviously, they are influenced by a particular local set of dietary patterns.

MINIMUM REQUIREMENTS

AYKROYD (continued): It would no doubt be an over-simplification to suggest that we could more profitably devote our attention particularly to the question of *minimum* protein requirements. But that indicates the general trend of my argument. Here we should recall that since most of the tables purporting to show protein requirements were produced, a great advance has been made in the whole subject; this is, quite simply, that the existence of specific and widespread disease due to protein deficiency has been recognized. We now know something about the protein content of diets eaten by children who develop protein malnutrition, and about the quantities and kinds of protein-rich foods which have to be given to these children to treat them effectively. At a later stage in our meeting we shall discuss this further. An essential practical problem is to determine the nature and amount of the protein-rich supplements—milk, fish, soya-bean preparations and so on—which should be added to a basic cereal or starchy diet in order to prevent protein malnutrition, or, in other words, to achieve some *minimum* standard of adequacy. There is some evidence, for example, that protein malnutrition tends to arise when foods containing less than 2 g. of protein per 100 calories constitute the staple diet. That would correspond, in the case of the hypothetical child of 2 years weighing 10 kg., to 2 g. per kg. of body weight.

I believe it will be advantageous to approach the whole problem with those concepts in mind and with reference to the existing situation in a great part of the world. Here I may be following Voit's fallacious reasoning, though in the opposite direction. But I believe that in following this line, effective use could be made of recent knowledge of amino-acid requirements and of the amino-acid composition of foods; in fact, that knowledge would be more valuable when this approach is adopted than it would be in a search for some hypothetical optimum. Another result might be to soften or remove the time-honoured distinction between animal and vegetable protein. Possibly some of the extensive knowledge of protein requirements arising out of livestock feeding could also be applied, although, of course, the object of the animal breeder is to secure maximum increase in weight in a minimum period of time, and the social and economic backgrounds of children and pigs differ fairly widely.

TERROINE: I repeat that unless we define our minimum on a sound physiological basis such as nitrogen balance our approach will remain empirical. The whole question depends on fixing the value of this minimum. There does not seem to be any difficulty about this in the adult, since all workers throughout the world seem to be in agreement. The most recent data on the minimal amount of protein necessary to produce nitrogen balance in the adult are those of Stare and his co-workers,[4] published in 1946, which are in exact agreement with the values obtained by Rubner and his school in the first decade of the

century. All workers agree that if proteins of high biological value are used, an intake of 25 g. a day is enough to produce nitrogen balance in the adult. Admittedly this only holds if the caloric needs are adequately covered.

Now the figures for protein requirements which have often been put forward in the past, and which Dr. Aykroyd has just mentioned, are for the most part much higher than this. Must we therefore suppose that the position of the nutritionist is quite different from that of the physiologist? Does this mean, as has often been said, that the minimum for health is much higher than the physiological minimum? If this is the case we must say so clearly, and define the value of the minimum for health and the basis on which it has been established.

WATERLOW: The criterion mentioned by Professor Terroine for the physiological minimum in adults is nitrogen balance. I suggest that this criterion is inadequate because a person may be in balance on a low intake as well as on a high intake.

KING: We might defer for a while consideration of what is meant by the term "requirement" and the criteria by which it is defined until we have more facts before us. Dr. Aykroyd raised the question of determining protein requirements in terms of amino-acids. I shall therefore ask Dr. Rose to summarize for us a classical chapter in the history of the search for a definition of protein requirements, at least in normal adults. This work provides some of the fundamental data which, as Professor Terroine has emphasized, are essential for a logical approach to the problem.

Amino-acid Requirements of Adults

ROSE: The work which I wish to summarize represents the results of a programme which has been under way for some twelve or thirteen years. It deals with only one phase of the problem that is before us, namely, the *minimum* amino-acid requirements of adult men. The methods which we have used have been described in detail,[5, 11] and it may only be necessary here to say that the subjects were young adult males who were normal as far as anyone could determine. They were graduate students in biochemistry, many of them my own students. They were as interested in the outcome of the experiments as I was and we can therefore have complete confidence in the part they played in the conduct of the experiments.

The diets contained, in place of proteins, mixtures of highly purified amino-acids which were taken in solution, flavoured with filtered lemon juice and sucrose. Most of the calories were furnished in the form of wafers which were made in the laboratory from corn starch, an inorganic salt mixture of appropriate composition, a small amount of corn oil to furnish highly unsaturated fatty acids, sucrose, and butter fat which had been melted and centrifuged to remove particles of protein.

Many difficulties were encountered, particularly in the earlier stages of the work, because we did not realize at the time that such diets necessitate rather high caloric intakes. This fact may introduce a question in your minds, I am sure, as to the general applicability of the results. It has been established, however,[16] that diets containing mixtures of amino-acids definitely require higher caloric intakes than similar rations containing the same quantity of nitrogen in the form of casein. No logical explanation of this can be offered at the present time.

ESSENTIAL AMINO-ACIDS FOR MAN

ROSE (continued): At first we undertook to determine how many of the amino-acids are required by man. Earlier, we had demonstrated that the growing rat requires ten: valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophan, lysine, histidine and arginine. Mixtures of these ten were used first in our human experiments. By dropping these out of the diets one at a time it was shown that, for the maintenance of nitrogen equilibrium in *adult* man, the first eight of the above list are indispensable components of the food.[5-9] On the other hand, adult man differs from the growing rat in that histidine and arginine are not required for nitrogen balance. We were very reluctant to believe that histidine is not necessary. Indeed, we repeated our experiments over and over again. Always the results were the same with respect to this amino-acid. More than fifty different individuals have now been maintained in nitrogen equilibrium without histidine. That arginine is not required was not surprising since we knew that adult animals frequently can synthesize this amino-acid at sufficient rates to meet their anabolic needs.

INDIVIDUAL AMINO-ACID REQUIREMENTS

ROSE (continued): Having established that eight amino-acids are necessary dietary components, we next undertook to determine quantitatively the requirements of adult man for each (*cf.* [11-19]). Originally it was hoped that for each amino-acid two quantitative tests upon different individuals would suffice. Subsequent events proved this assumption to be incorrect. Variations in the minimal requirements were observed from one subject to another, despite the fact that the findings in a given individual could be duplicated exactly, even though an interval of 6 to 8 months elapsed between the experiments. In order to learn the range of these variations in so far as possible, several experiments were made with each of the eight essential amino-acids.

1. *Lysine*

A typical experiment, which has recently been published,[11] to find the lysine requirement is summarized in Table 1. Starting with a diet that contained 10·10 g. of total nitrogen, and 1·2 g. of L-lysine, given as the monohydrochloride, the data show that the subject came into positive balance. It will be noted that the average daily retention of nitrogen for the 7-day period was 0·59 g. The

TABLE 1

L-lysine requirement of man

Period (days)	Initial body weight (kg.)	Daily N intake (g.)	Average daily N output		Average daily N balance (g.)	L-lysine* given (g. per day)
			Urine (g.)	Faeces (g.)		
7	69·4	10·10	8·52	0·98	+0·60	1·2
4	70·3	10·10	9·74	0·87	−0·51	0·6
7	70·8	10·10	9·00	0·72	+0·38	0·8
6	71·2	10·10	9·00	0·76	+0·34	0·7

* The lysine was administered as the monohydrochloride, but for convenience the daily dosage is expressed as the free amino-acid.

(Taken from original, Table II, Rose *et al.*, *J. biol. Chem.*, **214**, 581, by kind permission of the publishers.)

lysine intake was then dropped to 0·6 g. daily, since in a previous experiment upon another subject the latter quantity had proved to be sufficient. It will be observed that the individual went into negative balance. Increasing the lysine intake to 0·8 g. induced a positive balance, which persisted when the daily dose was reduced to 0·7 g. In most cases we have not attempted to determine the minimum requirement of any amino-acid more closely than to the nearest 0·1 g. per day. There are two exceptions to this. With tryptophan, one can measure the value to 0·05 g. with great ease. The same is true with isoleucine; 0·05 g. a day is enough to make a very noticeable difference in the state of the nitrogen balance. On the basis of the above data, we would conclude that 0·7 g. per day is the minimum L-lysine requirement of this particular individual.

WATERLOW: What degree of difference in balance do you regard as significant, or is it just a matter of whether or not it is positive?

ROSE: If you ask me what I think is the smallest significant change in nitrogen balance, I must admit that this is difficult to define. We have always assumed that the lowest intake which is capable of inducing a distinct, consistently positive balance, as measured over a period of several days, should be regarded as the minimum. I feel that with the experience we have had and with the care we have taken we can measure the true state of the nitrogen balance very closely.

These experiments with lysine are an example of the way in which we have tried to determine the requirements of each of the essential amino-acids. Our results are summarized in Table 2.

TABLE 2

Summary of amino-acid requirements of man

All values were determined with diets containing the eight essential amino-acids and sufficient extra nitrogen to permit the synthesis of the non-essentials.

Amino-acid	No. of quantitative experiments	Range of requirements observed (g. per day)	Value proposed tentatively as minimum (g. per day)	Value which is definitely a safe intake (g. per day)	No. of subjects maintained in N balance on safe intakes or less
L-Tryptophan ..	3*	0·15–0·25	0·25	0·50	42
L-Phenylalanine	6	0·80–1·10†	1·10	2·20	32
L-Lysine ..	6	0·40–0·80	0·80	1·60	37‡
L-Threonine ..	3§	0·30–0·50	0·50	1·00	29
L-Methionine ..	6	0·80–1·10‖	1·10	2·20	23
L-Leucine ..	5	0·50–1·10	1·10	2·20	18
L-Isoleucine ..	4	0·65–0·70	0·70	1·40	17
L-Valine ..	5	0·40–0·80	0·80	1·60	33

* Fifteen other young men were maintained in nitrogen balance on daily intakes of 0·20 g., though their exact minimal needs were not established. Of the 42 subjects maintained on the *safe* level of intake, 33 received 0·30 g. daily or less.

† These values were obtained with diets which were devoid of tyrosine. In two experiments, the presence of tyrosine in the food was shown to spare the phenylalanine requirement to the extent of 70 to 75 per cent.[18]

‡ Ten of these subjects received daily intakes of 0·80 g. or less.

§ In addition to these 3 subjects, 4 young men received rations containing 0·60 g. of L-threonine daily and 16 others received doses of 0·80 g. daily. No attempt was made to determine the exact minimal requirements of these 20 individuals, but all were in positive balance on the doses indicated.

‖ These values were determined with cystine-free diets. In three experiments, the presence of cystine was found to exert a sparing effect of 80 to 89 per cent upon the minimal methionine needs of the subjects.[17]

2. Phenylalanine and methionine

The phenylalanine requirements were determined with tyrosine-free diets, and the methionine requirements were measured with cystine-free diets. It becomes of considerable interest to know whether or not the presence of tyrosine and cystine can reduce the needs of the organism for phenylalanine and methionine respectively. When the requirement for phenylalanine in the absence of tyrosine had been measured, L-tyrosine was added to the diet and the phenylalanine requirement determined again (*cf.* [18]). 1 g. of L-phenylalanine maintained positive balance in the subject under study, but 0·9 g. was not quite enough. According to the criteria used, 1·0 g. was the minimum requirement of the subject in the absence of tyrosine. Without altering the 0·9 g. level of L-phenylalanine, 1·1 g. of L-tyrosine, which is equivalent to 1·0 g. of phenylalanine, was added to the daily ration. Immediately, a strong positive balance occurred. The L-phenylalanine intake was then progressively decreased while maintaining the tyrosine content of the food at a constant value. In these circumstances 0·3 g. of phenylalanine was the minimal amount capable of maintaining nitrogen balance. Thus, tyrosine exerted a 70 per cent sparing effect upon the phenylalanine needs of the organism. In another subject the sparing effect was 75 per cent.

Similar experiments were done to show the sparing effect of cystine on methionine requirements (*cf.* [17]). This is particularly important in the problem before the conference, since in some areas of the world the methionine intake seems to be the limiting factor. Daily intakes of 1·0 and 0·9 g. of methionine were sufficient on a cystine-free diet, but 0·8 and 0·7 g. doses gave slightly negative balances. One would regard 0·9 g. then as the minimum requirement of the subject.

During the next period the introduction of 0·81 g. of L-cystine, equivalent to 1 g. of methionine, again gave a strongly positive balance. The methionine intake was then progressively diminished to zero; at this point a negative balance was produced which tended to increase the longer the experiment was continued. However, 0·1 g. of methionine daily maintained positive balance. Therefore, starting with a requirement of 0·9 g., the presence of cystine reduced the methionine need by eight-ninths, or 89 per cent. In two other subjects the sparing effect of cystine was found to be 80 and 87 per cent. These data indicate clearly that consideration must be given to the cystine content of diets in those areas of the world in which methionine seems to be a limiting dietary component.

Finally, we have also shown that the human organism is not able to utilize the D-isomers of isoleucine, leucine and valine.

EXPERIMENTAL BALANCE PERIODS

DEAN: Were your balance periods consecutive or do you have a rest period in between?

ROSE: They are consecutive.

DEAN: So you are taking a subject who has been in negative balance and depleted, and therefore abnormal, and you try to make him normal by giving him a higher amount of the test amino-acid? Do you not think this might affect the validity of your experiments?

ROSE: No, I do not. The periods of negative balance were of short duration. We have made many experiments to determine whether changes in the blood picture resulted from this, and have never been able to find any. If any depletion

occurred, it was a very slight one. If one omits the first day following a dietary change, the positive balance becomes even greater than it is here represented. I agree, of course, that the state of depletion would influence the magnitude of the positive balance. We found that when one removes a given amino-acid completely from the diet for 5, 6 or 7 days, and then restores it to the diet, a very strong positive balance occurs for a time which slowly drifts back to a slight one, as the subject compensates for the nitrogen lost during the earlier period. But when only a slight change in the intake is made, as in the quantitative measurements being described, the degree of depletion is not enough to cause a marked retention when the dosage is again increased.

TERROINE: Your observation, that when you introduce a certain essential amino-acid into the organism the nitrogen balance is at first very high and gradually falls to what is regarded as the ordinary level, is only an example of what was observed years ago by Bischoff and Voit, the founders of the laws of nitrogen balance. They showed that when one passes from one level of dietary protein to another there is a latent period before nitrogen balance is established at the new level. If you start with a very low intake, either of proteins as such or of a mixture of amino-acids, and then change to a much higher intake, there will be a period of nitrogen retention before balance is established at the new level. This retention will be particularly large if the initial protein intake is not enough to produce balance. Conversely, if you change from a high to a low intake, there will be a temporary loss of nitrogen. As is well known, these phenomena probably result from changes in the protein reserves of the organism and particularly in the amount of liver protein.

STARE: I think most of your experimental periods lasted for about a week. If you reduce any one of the essential amino-acids to a level at which the subject goes into slight negative balance, and keep it at that level for 2 weeks or 3 weeks instead of 1 week, will the slight negative balance gradually be reduced, so that the subject comes into balance?

ROSE: No, it will continue and gradually increase—that is, become more strongly negative, if you carry on long enough.

DARBY: If you do not leave the essential amino-acid out of the diet entirely, but give a slightly smaller quantity than the normal requirement, does the organism eventually adapt to that?

ROSE: No, we have never found that. There are many difficulties involved in experiments of this type; you can't keep these young men on a deficient diet like this indefinitely, because they begin to manifest symptoms associated with amino-acid deficiencies, particularly loss of appetite. They must then force themselves to eat the food, and if they keep that up long enough, they vomit and spoil the experiment. They also get very irritable and threaten to quit. These symptoms of appetite failure and irritability are much more profound with some types of amino-acid deficiencies than with others. Deficiencies involving valine and isoleucine are particularly prone to bring about loss of appetite, nervousness in general, and a sensation of fatigue. The symptoms appear very promptly, despite the fact that in many of our experiments the dietary changes were made without the knowledge of the subjects. We have spoiled many experiments by trying to keep the subject on an inadequate diet for too long a period.

DEAN: I think Darby's suggestion is of great importance to those of us who work in under-developed countries, because we suspect that many of the people we are dealing with are chronically short of protein and may have made some

adjustment. I have often considered the possibility of doing these experiments in a different way, by giving a diet low in natural protein, a diet of which we had the complete analysis, and then adding the test amino-acids. Do you think that we would get the same answer as with an entirely synthetic diet?

ROSE: That is another way, which has both advantages and disadvantages. The problem of availability of the protein would complicate the interpretation.

CALORIE INTAKES

TERROINE: It is very surprising that you have to give such a high caloric intake—about 4,400 calories per day. What is the reason for this? Does the fact that the nitrogen requirements are supplied by a mixture of amino-acids for some reason result in an increased energy expenditure? It is possible, for instance, that when amino-acids are given in pure form, and so penetrate the tissues very rapidly, there may be an increase in specific dynamic action. When proteins are fed as such, amino-acids must enter the tissues more slowly, according to the rate at which they are liberated by digestion.

ROSE: I have no logical explanation for the high caloric requirement in our experiments. I agree with Professor Terroine that amino-acids may be absorbed much more rapidly when taken in the free form, but I do not know whether this could cause a change in specific dynamic action. Moreover, when consumed in the form of proteins, in the course of digestion they may be liberated in just the order in which they are most efficiently utilized by the tissues after absorption. Perhaps that has something to do with the difference in caloric requirement. We have determined the output of amino-acids in the urines of several subjects receiving the mixtures and there is very little spillage. Furthermore, the amino-acids found in the urines of subjects on our diets are predominantly those that have been administered in the racemic form, namely, valine, isoleucine and threonine. Chromatographically, you can show that these urines constantly contain far more valine, isoleucine and threonine than other amino-acids. This is presumptive evidence that the urinary amino-acids are predominantly the D-isomers. Thus, spillage will not account for the higher caloric requirement.

The most logical view, it seems to me, is that amino-acids are absorbed at different rates when given in the free state and when liberated by the process of digestion, and that possibly the *order* of liberation has some relationship to the synthesis of body proteins.

GYORGY: Don't you think there is a possibility that peptides may be absorbed as such, and in some way give different results from free amino-acids?

ROSE: I cannot exclude that possibility. I think it quite likely that peptides are absorbed, but what part they play, if any, is not clear. I have pointed out that the behaviour of peptides towards micro-organisms has been found to differ from that of the amino-acids which they contain.[10] This is a factor that must be taken into account. I do not claim that our data explain *everything*. It is one line of approach to the problem of protein requirement. Other factors are involved, and at the moment we don't know what influence they may exert. Every time I have spoken of these minimal requirements, you will find that I have referred to them as "tentative". These are the *tentative* minimal requirements for the maintenance of nitrogen equilibrium in *normal* adults.

MAYER: If it is a matter of absorption, would it make a difference whether the diet, apart from the amino-acids, is high in carbohydrate or high in fat?

ROSE: Probably it would. However, we have used very uniform diets from that point of view. Except at the very beginning of our experiments, when the

possible effects of carbohydrates and fats were not fully appreciated, we have used diets in which the ratio of calories derived from carbohydrate to those furnished by fat was maintained at 2·6.

WATERLOW: How many times a day do you feed the amino-acids?

ROSE: Three times a day. The subjects take one third of the day's food at each meal-time. The amino-acid solutions are sipped while the balance of the diet is being consumed.

WATERLOW: Quite apart from spillage in the urine, there is evidence, I think, that the more you flood the body with amino-acids, the more metabolism is turned towards deamination and oxidation rather than to synthesis.

SÉNÉCAL: What happens to the other amino-acids when one is deficient?

ROSE: I suspect that they are metabolized, oxidized, and the end-products excreted. You can't build proteins unless you have all of the necessary building blocks. If you take out one of them, then the rest become largely useless. They must be metabolized, because the urea output increases during amino-acid deficiencies.

SCRIMSHAW: I think there is evidence that protein can sometimes be formed from incomplete amino-acid mixtures. Albanese[20] recently showed that under certain circumstances plasma protein can be synthesized on a lysine-poor diet.

ELVEHJEM: I don't know if that is correct.

DARBY: What happens to the calories, or to the subjects, rather, on this large caloric intake? Do they gain weight?

ROSE: It depends. All subjects gain a little weight, but some gain quite markedly if they are the type of person that tends to put on weight under normal dietary conditions.

TERROINE: If they do not gain weight they must be expending more energy. Have you made measurements to see if this is so?

ROSE: No. We keep the activity as nearly constant as we can. The subjects are working in the laboratory and are not permitted to change their activities.

PLATT: Was the high food intake that you give in these experiments more or less what they were accustomed to? If so, there does not seem to me to be any problem.

ROSE: I have no information about the previous caloric intake of the subjects, but I doubt if it would have been as high as they received during the experiments.

MAYER: Does it mean, then, that they step up their non-productive activities? For instance, you said they often become nervous; do they make a lot of unnecessary movements? Surely, if someone gets 4,400 calories and is fairly sedentary, something has got to give?

DARBY: If they do not gain weight they might either have a shift in water balance or else be excreting some metabolic products in an incompletely oxidized form.

ROSE: I doubt that a change in water balance occurs.

DARBY: But how can they possibly take in an extra thousand calories a day over a long period of time without either showing a gain in weight or failing to oxidize their food?

ROSE: They *must* oxidize it. It comes out as carbon dioxide and water.

TERROINE: Dr. Mayer has suggested that this energy-expenditure could result from a transformation in the body of carbohydrate to fat. If that were so, some evidence could be obtained by measurement of the respiratory quotient. Have you done this?

ROSE: No, but I made two or three measurements of the basal metabolic rate early in the experiments. Since there was no correlation between the amino-acid requirement on the one hand and body weight, body surface, or creatinine output on the other, we hoped that we might find some correlation with the basal metabolic rate. The first few results were not encouraging, since perfectly normal values were obtained. However, not many experiments of this sort were done; therefore this is another aspect of the problem that somebody might undertake.

SCRIMSHAW: This failure to gain weight is very much like that found in cases of marasmic kwashiorkor. During many weeks of treatment, with relatively enormous caloric and protein intakes, they may show little or no gain in weight.[21]

MAYER: It does raise the point that tremendous differences of body composition could conceivably occur, even though variations of weight may not arise. Ohlson[22] has just shown that when women are put on low caloric intakes in order to reduce weight, some gain protein during the reduction diet as they lose weight, and others go into negative nitrogen balance. Now, this is a sort of reverse of your experiment.

ROSE: I haven't analysed these boys, so I don't know. But don't misunderstand me. Some of them *do* gain weight, although others gain very little. I should like to make it clear that I used the higher caloric intake merely because I found it impossible otherwise to get these men into balance without a very long fore-period. Actual comparisons between the caloric requirement on proteins and mixtures of amino-acids showed that it was much easier to bring a man into balance on a given amount of nitrogen in the form of proteins than in the form of amino-acids.

If you examine some of the other experiments in the literature, in which low protein diets were used, such as those of H. H. Mitchell,[23] in which he fed low protein diets to young women to establish the lysine requirement, you will find that it was necessary for him to administer as much as 50 calories per kg. of body weight. This is true also of Murlin's experiments, in which he fed proteins supplemented with amino-acids.[24] Ordinarily the energy intake was placed at 45 calories per kg., but, as I have quoted elsewhere,[11] "when in individual cases it became apparent that this allowance was not sustaining body weight, it was raised to 49 calories per kg. or sometimes higher, by addition principally of carbohydrate".

DEAN: How did you determine the level at which to start the test amino-acids in your experiments?

ROSE: The starting levels were based purely on a guess. We had no information when we began these experiments in 1942 about what the human amino-acid requirements might be. We made a rough calculation of the distribution of amino-acids that one might expect if one consumed 10 g. of nitrogen in the form of casein. We rounded those figures, and used them as the initial intakes. They were much higher than we needed; as we observed the minimum for a given amino-acid, we invariably reduced the quantity of that amino-acid in subsequent experiments in order to save money. The diet that we used at the start, with these relatively high intakes, cost $20 per man per day. Therefore, we were very anxious to save amino-acids as rapidly as we could.

VARIATION IN DIFFERENT SUBJECTS

ROSE (continued): We began with an intake of 1·85 g. of tryptophan a day, just to make sure that we had enough, and then, after we found that two

subjects required 0·15 g. we dropped the level to 0·2 g., thinking that we were supplying a little excess. Seventeen different subjects were maintained in balance with 0·2 g. or less, until we encountered a man who required 0·25 g. Then we raised the intake in subsequent experiments to 0·3 g. and later to 0·5 g., just to make sure that it could not be a limiting factor while we were measuring the requirements for other amino-acids. There was no significance attached to the initial intake, except that we were trying to adopt one that would be adequate: that is, enough to maintain nitrogen balance.

The figures I have just quoted illustrate the variation between subjects. In most experiments 5 or 6 subjects were used. However, with isoleucine only 4 experiments were carried out because the results were so remarkably constant. In the case of tryptophan and threonine a large number of subjects received intakes only slightly above the minima and were maintained in balance. I would agree with anyone who wishes to criticize the data from the point of view of the number of subjects. It would be desirable to use scores of individuals with each amino-acid. However, it is not feasible to carry out a tremendous number of human experiments such as one would expect to do in animals.

The range of values is indicated in Table 2 (page 7) for each amino-acid. The range for leucine was the greatest of all. The range for isoleucine was the least; the others were intermediate. Note, however, that both valine and lysine had a range of 100 per cent, or from 0·4 to 0·8 g. Ranges such as these make it difficult to decide on the best way of expressing the minima. If one had enough subjects, a mean value with a probable error would be satisfactory. I doubt if this procedure would be worth much when the number of experiments is small. Consequently, we have chosen, throughout, to regard the highest observed value as the "tentative" minimal requirement. Furthermore, we recognize that individuals other than those tested might have still higher requirements. In order to allow for this possibility, diets designed to prevent amino-acid deficiencies should contain still larger quantities of the essential amino-acids. We have proposed that twice the highest observed minimum be used for this purpose and be designated as the "safe" intake. The "safe" intake of each amino-acid is shown in the fifth column of Table 2. Statistically, one can demonstrate that the probability of anyone having an amino-acid requirement in excess of the "safe" intake is extremely small. The adequacy of the "safe" intakes has now been substantiated in a fairly large number of individuals, as shown by the figures in the sixth column of Table 2.

We were unable to find any correlation between the body weights of the subjects and their requirement for a given amino-acid. This does not imply that the mass of active protoplasm has nothing to do with the amino-acid requirement, but only that other unknown factors also affect the body's needs.

DEAN: Did you calculate the amounts of various amino-acids that your subjects had been obtaining from their usual diet?

ROSE: No. It would be very difficult to make accurate calculations such as you suggest. I think the food intakes would be very variable and might not correspond at all to the figures I have given you.

DEAN: I just wonder whether they might be related to these variations in requirement that you found.

ROSE: I have no evidence on that, but I don't think so.

KING: I think you found, however, that when these individuals were tested again, they nearly always showed their original pattern.

ROSE: Yes. There was variation from one subject to another: but in the same subject we could repeat the experiment and come out with exactly the same answer.

DEAN: After a period of years or months?

ROSE: Months; we didn't try it over a period of years; but as much as 6 to 8 months elapsed between some tests. It is very improbable that before these tests the same intake of protein was consumed.

PLATT: Does your work with rats throw any light on the questions we have been discussing—the need for a high caloric intake, and the varying requirements of different individuals? I recognize, of course, the limitations of arguing from one species to another.

ROSE: We have never determined the caloric requirements of the rat in any of our experiments. They are fed *ad libitum*, and we notice the drop in appetite with a deficiency, just as we do in man, but we have no data on the caloric intake of the animals.

PLATT: Does the rat's requirement for a single amino-acid vary as it does in man?

ROSE: There are no obvious variations, but it is difficult to say positively since one is dealing with such a small animal. On the basis of body weight, variations in the rat, if they occur, would be very small. What I have generally done with animals is to use a rather large group. In a recent experiment I think we used over 300 rats to determine the minimal tryptophan requirement. Variations fade out because the mean of a group receiving one level is compared with the mean of a group ingesting another level. There are individual variations in the *gains* made by members of the same group, and these *may* reflect differences in minimal requirements.

I have been much interested in the recent work of Dr. Roger Williams[25, 26] in which he calls attention to all kinds of variations that one may observe in man. He is rendering a real service, I think, in emphasizing just the reverse of what most of us have been doing. We have been *standardizing* man, and now he is destandardizing him. He thinks that there is no such thing as a "constant", "uniform", or even "normal" man.

FREMONT-SMITH: No matter how magnificently a basic study is done—and this certainly is an example of one—it never can answer all the questions for all men. It inevitably suggests many other things that other people would like you to do, and that means, of course, that they should go and do some of these additional experiments themselves.

ROSE: I heartily agree with you.

NITROGEN FOR NON-ESSENTIAL AMINO-ACIDS

ROSE (continued): There is, however, one other problem I want to tell you about. The last question we tackled was an approximate determination of the amount of nitrogen required by man for the synthesis of the non-essentials.[27] If one were to administer a diet containing the minimal levels of the eight essentials only, one would not expect the body to be able to maintain nitrogen balance until an extra supply of utilizable nitrogen is added, which the cells could employ for the synthesis of the non-essential amino-acids. Throughout our experiments, extra nitrogen has been included in the form of glycine and urea, both of which can be utilized by the rat for synthetic purposes.

FREMONT-SMITH: When you say "essential", do you mean essential for nitrogen balance?

ROSE: Yes, I mean amino-acids that must be consumed in a preformed state, as contrasted with those that can be manufactured by the body—in other words, essential components of food. All amino-acids are, of course, essential for tissue formation.

Now, the method here was to start with a diet containing 10 g. of nitrogen, including twice the minimal requirements (safe intakes) of the eight essential amino-acids, and the remaining nitrogen in the form of glycine and urea. With this ration, nitrogen balance was obtained promptly. All the urea, representing 2 g. of nitrogen, was then excluded from the diet. Four days were allowed for adjustment. On each of those days the nitrogen output in the urine was determined, so that we could tell whether or not the subjects were getting back into nitrogen equilibrium. This was followed by a 6-day period on the 8 g. intake. Nitrogen balance was again maintained. Glycine equivalent to 2 g. of nitrogen was then removed from the food, and another adjustment period of 4 days was allowed. The 6-day observation period again showed that nitrogen balance was maintained on the 6 g. intake, and similarly with 4 g. However, 3 g. yielded a negative balance, which was reversed by raising the intake to 3·5 g. Thus, 3·5 g. of total nitrogen was the minimal quantity that would maintain this subject. Exactly the same answer was obtained in a second subject. These two subjects weighed 67 and 82 kg.

The question then arises, what were the sources of the 3·5 g. of nitrogen? Examination of the diet shows that the so-called effective amino-acids furnished 1·42 g. of nitrogen. By effective I mean all the L-amino-acids, D-methionine,[15] and 0·5 g. of D-phenylalanine.[13] Together these represent a surprisingly small quantity of nitrogen. On several occasions in the past I have suggested that a mixture of amino-acids, the composition of which is adjusted to the need of the organism for each component, might be the most efficient form of nitrogen yet devised. Our findings seem to confirm this idea. Bear in mind, however, that the quantity in question is not a minimal figure, since the food contained *twice* the minimal requirements of the essentials. If one were to determine in the same individual the minimal need for each essential a figure smaller than 1·42 g. would be obtained.

The ineffective amino-acids—by which we mean the nitrogen of the D-isomers of valine, isoleucine and threonine and 0·7 g. of D-phenylalanine—accounted for 0·52 g. of nitrogen. Probably this was not utilized. Glycine furnished 1·21 g. of nitrogen, and the other components of the food contained 0·35 g. of unknown nitrogen, most of which was derived from the starch of the wafers. From these data, and from the range of minimal requirements recorded in Table 2, one may calculate that the nitrogen needed for the synthesis of the non-essentials probably lies between 2·28 and 2·55 g. daily.[27]

GYORGY: The extremely low nitrogen intake that you achieved is most surprising. An intake of 3·5 g. of nitrogen a day for a subject weighing some 70 kg. works out at about 0·3 g. of protein per kg. per day.

ROSE: Yes; however, the purpose of these experiments was not to determine the minimal intake of nitrogen, but to measure approximately the amount of nitrogen needed for the synthesis of the non-essential amino-acids. You can get lower intakes than I have described.

CONCLUSION

TERROINE: Professor Rose's beautiful experiments provide a striking confirmation of earlier work, carried out not with amino-acids but with proteins

As regards the total requirement, Dr. Rose has arrived at a figure of 3 g. of nitrogen a day and sometimes even less. This is precisely the figure given by Rubner and his colleagues, Thomas and Zeller, from experiments made over quite long periods. I recall that Hindhede in 1914 maintained nitrogen equilibrium in an adult subject for 50 days on a daily nitrogen intake of 3·48 g.

The second important point concerns the relation between the total requirement of essential amino-acids and that part of the nitrogen requirement which can be supplied by glycine. This represents the fundamental distinction which I made in 1930 between what I called the specific requirement (total of essential amino-acids) and the non-specific requirement, which can be provided by any form of nitrogen (any amino-acid, amide or organic ammonium salt).

As regards the non-specific requirement, Dr. Rose states that if glycine is used it amounts to 40 per cent of the total nitrogen requirement. Now, in 1930 I showed in experiments with the pig that 44 per cent of the endogenous nitrogen expenditure could be covered by giving ammonium citrate.[28] There is therefore a remarkable agreement between our two figures.

ROSE: I am familiar with Rubner's experiments and also with results of Scandinavian workers.[29] They were rather prolonged experiments, and sometimes a considerable period of time was necessary for the establishment of nitrogen balance at the low levels. That is the reason why I emphasized that our experiments were not designed to determine the lowest total nitrogen intake but, rather, to get an approximate measure of how much was required for the synthesis of the non-essential amino-acids. Starting out with twice the minimal levels, you could cut that in two immediately, assuming that no subject had a requirement higher than those we had observed.

GYORGY: Your results and conclusions were drawn from balance experiments of relatively short duration. Can you guarantee that people can live on such a low protein intake for years? This is a question of great practical importance.

ROSE: No, I can't guarantee anything except what I have stated. You know, fifty years ago, one of my distinguished teachers, Professor Russell H. Chittenden, was quite a propagandist for a low protein diet. He showed that he could live on 35 to 40 g. of protein and get along perfectly well. He died in his 88th year, and was in perfect health until shortly before his death. It would be difficult to prove that his low protein diet was detrimental. I'll leave it to you to tell me whether he lived long because of his diet or in spite of it. The important point in Chittenden's experiment, I think, is the remarkably low intake one can ingest without coming to harm. Exactly the same thing applies here. One can reduce the intakes of amino-acids as shown, maintain nitrogen balance, and, at least for short periods, see no evidence of abnormality. Beyond this I have no information.

Finally, I appreciate, I think, just as fully as anyone else, the limitations inherent in our observations on the amino-acid requirements of man. They represent one type of approach to the problem. I certainly do not wish to over-emphasize their significance. Admittedly, exactly the same results might not be obtained with diets containing native proteins, although I doubt that marked divergence would be observed by the two methods. In our opinion the values reported cannot be far from the true minimal requirements of human subjects. We would much prefer to measure *optimum* intakes, if methods were available for doing so. All one can do at present is to determine the minima and then make a reasonable allowance for an excess of each amino-acid to take care of the optimum needs. One should bear in mind that these data are for

normal adult men, and do not relate to children at all. Furthermore, no one knows whether or not other factors, such as disease, may affect the amino-acid needs. Certainly one would expect that pregnancy and lactation would increase the requirements of females.

GYÖRGY: But we have to face the practical question of what is the protein requirement to be recommended in the world.

ROSE: My own feeling is that as a starting point in our recommendations we might aim at a protein intake which will furnish "safe" intakes of the essential amino-acids. Later these could be modified if found necessary. Of course on a body-weight basis more would be needed for the growing infant.

Amino-acid Requirements of Infants

KING: I think it would be useful now to hear from Dr. Holt about the work that he has been doing along the same lines in infants.

HOLT: The experiments which I shall show you were carried out by a somewhat different technique from that used by Dr. Rose. Also, they were done later, and we had the advantage that some of the amino-acids which had not been available to him except in racemic form were available to us in natural forms.

We studied babies on completely synthetic diets made of an amino-acid mixture, a malt-dextrin preparation, a hydrogenated vegetable oil, and a mineral mixture containing the known vitamins. The amino-acid mixture contained eighteen amino-acids, exclusively in their natural L-forms; it supplied the non-essentials as well as the essentials. Dr. Rose has shown that the non-essentials can be manufactured from glycine, but it seemed that one might get better data if one did not ask the body to do this synthesis and supplied these amino-acids individually. We adopted a basal amino-acid mixture based on analyses of breast milk, largely from the compilation of Macy *et al.*,[30] but modified in two minor respects by analyses carried out in our own laboratory by Cheung, Pratt, and Fowler.[31]

We first tested this diet to make sure of its adequacy in a premature infant—the most rigorous test we could apply. The growth curve of this infant on the synthetic diet followed the standard growth curve for his birth weight.

DEFICIENCIES OF INDIVIDUAL AMINO-ACIDS

1. Threonine

We then went on to study the deficiencies of one amino-acid after another. Figure 1 illustrates an experiment in which we omitted threonine entirely, and then stepped it up to various levels until we found what appeared to be the minimal amount of threonine that could support weight gain in an infant.[32] In a child one is at a disadvantage: one cannot use nitrogen equilibrium as an end point, because children, and small infants in particular, tend to retain nitrogen to some extent even on diets deficient in some essential amino-acid. Only exceptionally do they go into negative balance. We must concern ourselves with the degree of nitrogen retention and decide whether it is normal or subnormal. In this subject the figure shows the extent of the positive balances on the complete amino-acid diet containing 180 mg. of threonine per kg. This particular infant did go into negative balance when the threonine was dropped out. We increased his intake to 70 mg. per kg. and obtained satisfactory nitrogen

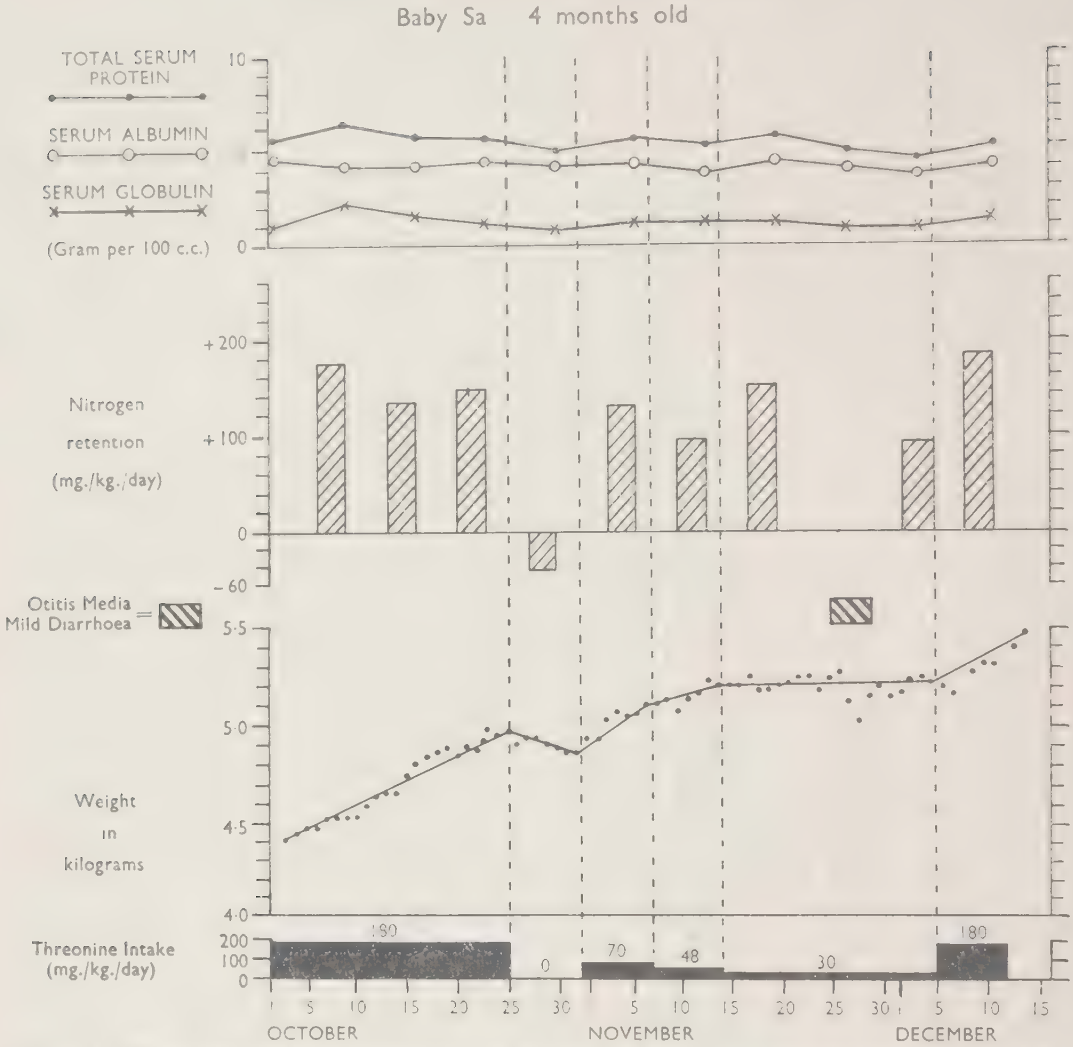

FIG. 1. The threonine requirement of the normal infant. (From the original of Figure 4, E. L. Pratt *et al.*, *J. Nutrition*, **56**, 241, by kind permission of the publishers.)

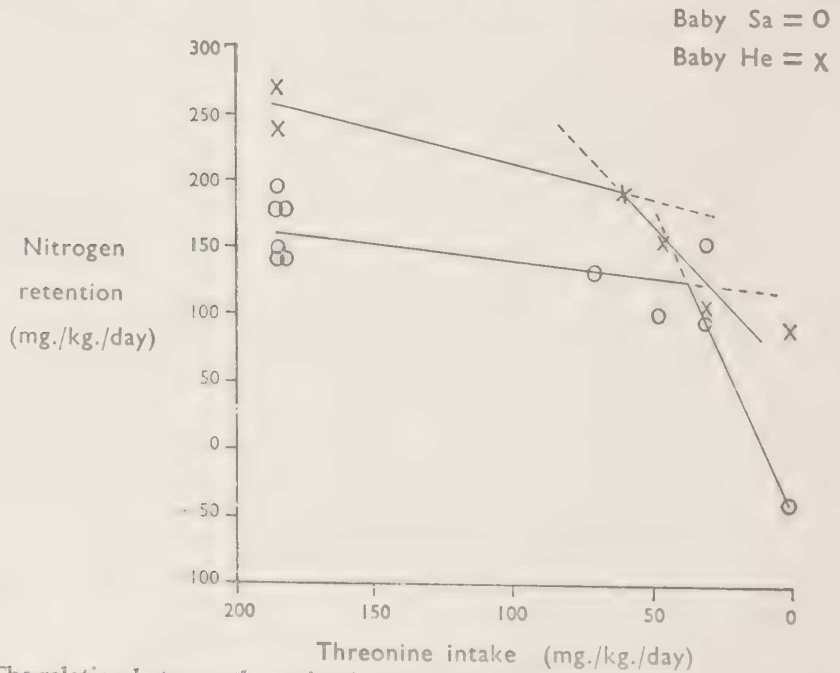

FIG. 2. The relation between threonine intake and nitrogen retention. (From the original of Figure 3, E. L. Pratt *et al.*, *J. Nutrition*, **56**, 240, by kind permission of the publishers.)

retention. On reducing the intake to 48 mg. the adequacy of the diet appeared to be questionable; nitrogen retention seemed to be falling off, and the weight curve was not going up quite as fast. At 30 mg. per kg. we got a practically stationary weight and a further fall in nitrogen retention; we then returned to 180 mg. per kg.—the normal figure—and again obtained good nitrogen retention and weight gain. Our conclusion was that the threonine requirement of this child was somewhere between 48 and 70 mg. per kg. Other experiments with threonine have given similar results. On the basis of six subjects studied we have reached the conclusion that a threonine intake of 48 mg. per kg. or less was inadequate and that 60 mg. per kg. was adequate.

In Figure 2 are presented data on nitrogen retention plotted against threonine intake. There appears to be a break in the curve, at the point where the minimum requirement is reached. Whether this phenomenon will survive a larger series of observations, I do not know. Our data on the threonine requirement are summarized in Table 3.

TABLE 3

Threonine requirement of infants[32]

Subject	Threonine intake (mg. per kg. per day)	
	Inadequate	Adequate
He	30	45
Sa	48	70
St		47
Ne	59	87
Mo-A	45	60
Ca	44·5	
de la R	45	
Ro	0	

(From original of Table 2, E. L. Pratt *et al.*, *J. Nutrition*, **56**, 244, by kind permission of the publishers.)

2. *Phenylalanine*

Figure 3 shows some data on the requirement of phenylalanine[33]; the normal intake on our basal diet based on breast milk is 193 mg. per kg. As we reduced the phenylalanine intake to 63 mg. per kg. we found a questionable but not very striking reduction in nitrogen retention. When we dropped the phenylalanine out entirely, there was a very sharp fall in nitrogen retention and a loss of weight. At 91 mg. per kg. normal weight gain was restored. At 61 mg. per kg. it seemed to fall off a little, although there was no reduction in nitrogen retention. Our conclusion here was that 61 mg. per kg. was probably too little, and that 91 mg. per kg. was an adequate intake of phenylalanine.

Our results from observations on the phenylalanine requirements of five other babies are summarized in Table 4.

The table confirms that 91 to 94 mg. per kg. may be taken as the minimal requirement of the infant. It should be noted that this is the phenylalanine requirement in the presence of tyrosine; we have not attempted to measure it in the absence of tyrosine.

TABLE 4

Phenylalanine requirement of infants[33]

Subject	Phenylalanine intake (mg. per kg. per day)	
	Inadequate	Adequate
He	61	
Sa	63	91
Mo-B	63	94
Du		64
Th		47
Ri		63

(From original of Table 1, S. E. Snyderman *et al.*, *J. Nutrition*, **56**, 258, by kind permission of the publishers.)

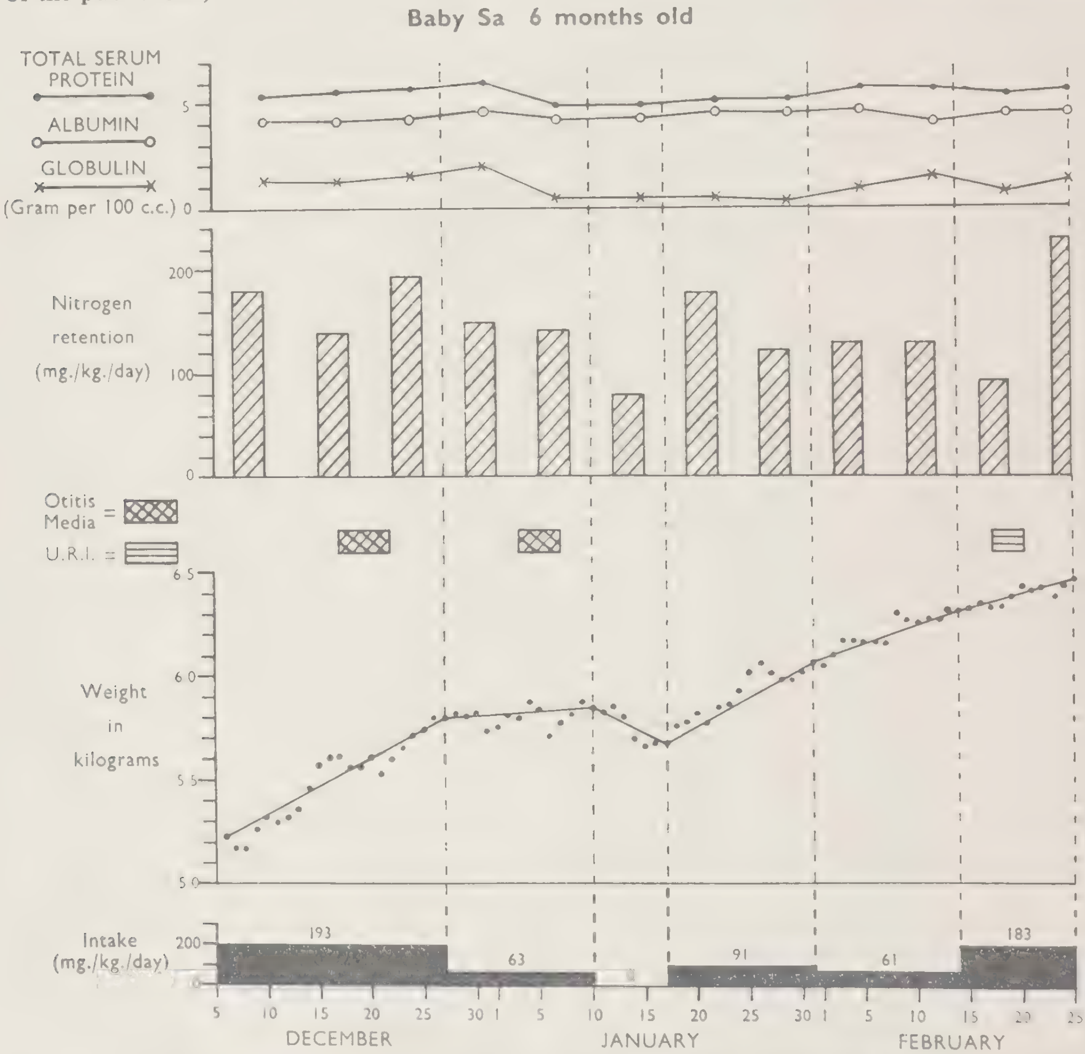

FIG. 3. The phenylalanine requirement of the normal infant. (From the original of Figure 3, S. E. Snyderman *et al.*, *J. Nutrition*, **56**, 257, by kind permission of the publishers.)

3. *Lysine*

Figure 4 illustrates a study of the lysine requirement in terms of the mono-hydrochloride of an infant. On our standard intake of lysine monohydrochloride of 265 mg. per kg. there is excellent nitrogen retention. Omission of lysine

caused a drop in nitrogen retention and in the weight curve. With an intake of 90 mg. per kg. the weight gain is subnormal and the nitrogen retention is also less than satisfactory. On increasing the intake of lysine monohydrochloride to 127 mg. per kg. excellent performance was again obtained, and this persisted

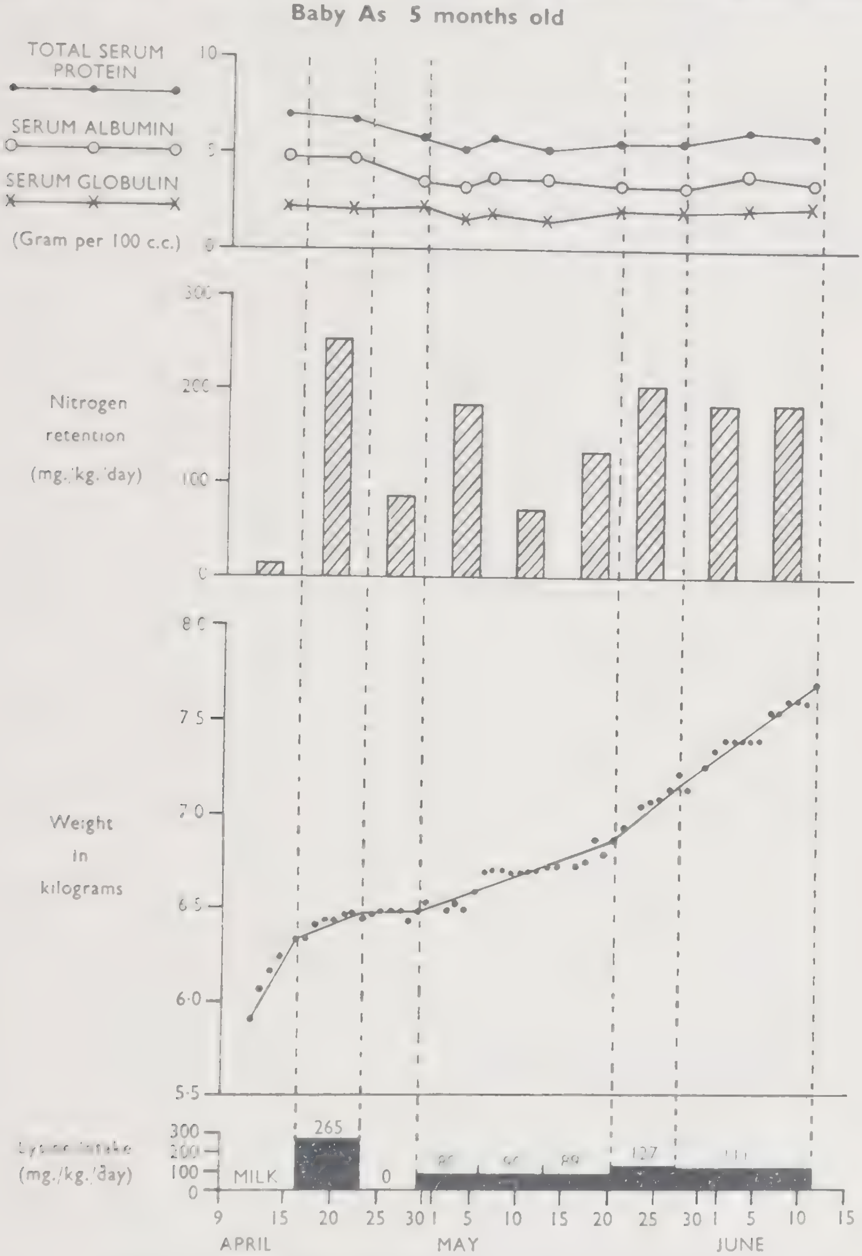

FIG. 4. The lysine requirement of the normal infant.

at a level of 111 mg. per kg. That, we feel, is about as close as we can come to the minimal requirement of this infant: 90 mg. per kg. is inadequate but 111 mg. per kg. is adequate. In a second study on another infant we reached the same conclusion—that 110 mg. per kg. represents the minimal requirement. This amount corresponds to approximately 96 mg. of lysine free base per kg. A third subject was tested only at this level (111 mg. per kg.) and was found to gain weight and retain nitrogen satisfactorily.

These studies contrast with some unpublished figures mentioned by Albanese,[34] which indicate that the lysine requirement of the infant on a diet of wheat

gluten, supplemented by lysine, is 170 mg. per kg. and may be as high as 200 mg. per kg. This apparently high requirement may be due to some inherent imbalance in wheat gluten which renders it unsuitable for a basal diet.

We have carried out studies of this kind on only three amino-acids—threonine, phenylalanine and lysine. The requirements of infants for tryptophan, isoleucine and methionine were surveyed a few years before by somewhat less accurate techniques, and some of these data need to be reassessed. One can also calculate minimal requirements, or rather an upper limit of minimal requirements, from the amino-acid content of low protein diets compatible with health. The most valuable data of this kind are those of Beach *et al.**[35] and of Swanson,[36] in which infants were fed for prolonged periods on breast milk which had been analysed. The available data on the amino-acid requirements of the infant are summarized in Table 5.

TABLE 5

Requirements of amino-acids in infants

Amino-acid	Observed intakes (mg. per kg.)				Minimal requirements (mg. per kg.)				
					Calculated		Observed		
	(1) Premature infants	(2) Full-term infants, early weeks	(3) Full-term infants, 1–4½ months	(4) Full-term infants, 4–5 months	(5) Calculated from intakes on various diets	(6) Calculated from intakes on low milk diets, full-term, 2–5 months	(7) Depletion studies on deficient protein diets	(8) Depletion studies on amino-acid mixture diets	(9) Suggested overall minimal requirements
Arginine	142	75	67	67	126	42			67
Histidine	55	29	26	21	63	24		33‡	26
Isoleucine	191	129	115		90	75	90		90
Leucine	365	239	210		425	135			210
Lysine	221	118	105	83	170	83	(170)*	90	105
Methionine	52	34	31	30	86	32	65†		31
Phenylalanine	162	95	85		169	61		90	90
Threonine	142	93	83		87	51		60	60
Tryptophan	52	32	29	31	30	16	30		30
Valine	206	134	120		161	80		64‡	120

* Not published, but referred to by Albanese.
† In the presence of cystine (85 in absence of cystine).
‡ Observations on one infant only.

(1) Data from Snyderman and Holt (unpublished).
(2) Infants fed pooled breast milk; protein intake 1·78 g. per kg. From Swanson.[36]
(3) Infants fed pooled breast milk; protein intake 1·60 g. per kg. From Swanson.[36]
(4) Data are averages of figures given by Beach *et al.*[35]
(5) Data from Albanese.[37]
(6) Data from Snyderman and Holt (unpublished).
(7) Data from Albanese, Holt *et al.*[38, 39, 40]
(8) Data from Pratt, Snyderman *et al.*[32] and Snyderman, Pratt *et al.*[33]

In many of these studies we followed the urinary excretion of the various amino-acids, using the column chromatographic technique of Stein and Moore. We quite consistently found a drop in the excretion of the amino-acid under study when we reduced the intake to zero. This was observed with phenylalanine and threonine, and probably occurs also with lysine.

We had hoped that in using a diet containing exclusively the L-amino-acids and one which provided the non-essentials as well as the essentials, we might be able to avoid the increase in caloric intake which Dr. Rose described in his

* Beach's data are calculated from nitrogen analyses, but a deduction (and I think an over-generous one) was made for the non-protein nitrogen of the intake, which makes all his figures low. He may be closer to the truth than other people but his figures are surely not comparable. Macy subsequently repeated analyses of breast milk in the same laboratory and found considerably higher values for many amino-acids. (L. E. Holt.)

experiments. We have not been successful in that. In some of our children we have had to add an excess of 25 per cent to the calories in order to establish normal nitrogen retention and weight gain. We are inclined to believe that specific dynamic action is increased by a diet in which all the amino-acids are supplied independently. Measurements of the amino-acids in the urine by column chromatography have failed to reveal any substantial loss of amino-acids on our experimental diet as contrasted with a milk diet.

ROSE: I should like to compliment Dr. Holt on this very interesting work, particularly since, as he knows, we have not always agreed on some aspects of amino-acid metabolism. I think he is perfectly justified in using the growth method rather than nitrogen balance, since, of course, the growing subject is always in positive balance. I am also interested to see that he finds a large variation in the phenylalanine requirement (47–90 mg. per kg.) very much as we found in the adult.

HANSEN: I think it is very interesting that when you cut out an essential amino-acid you still get a positive nitrogen balance and not a negative one, as in the adult.

HOLT: That seems to be particularly true of the young child. The younger the child, the greater the tendency for the balance to remain positive even when an essential amino-acid is omitted.

EFFECT ON APPETITE

HOLT (continued): I want to say one thing more. I think that we have been able with our diet to eliminate one phenomenon encountered by Dr. Rose in his experiments—a progressive failure of appetite. These children do not accept the amino-acid diet immediately—indeed children don't accept any change of diet readily at first—but with skilful nursing and coaxing they can be induced to take the diet, and do so with a reasonable degree of relish. Children will take the most extraordinary diets if they are properly conditioned. We have substituted tributyrin for butter fat in infant formulae. By changing the fat gradually we can get infants to take with relish an exceedingly bitter-tasting formula in which all the fat is supplied as tributyrin. The same is true of amino-acids. They will take these amino-acid mixtures, and continue to thrive for as much as 6 or 8 months, without showing any sign of failure of nutrition or failure of appetite. However, there is a defect in the synthetic diet which begins to show up at 6 or 8 months. I don't know what it is. At the end of that time there is a failure to gain weight and to grow, though the infants remain perfectly healthy and happy. We are trying to identify the nutritional factor concerned.

FREMONT-SMITH: Are they still in positive balance?

HOLT: We have no balance data on such children.

EFFECT ON BODY PROTEINS

ALLISON: May I emphasize something that seems to be very important in our work with dogs—that nitrogen balance is the algebraic sum of gains and losses from all the tissues of the body. It is possible therefore to have an animal in positive nitrogen balance and yet have some tissue proteins in negative balance. It is our belief that in the growing puppy or in the dog depleted in protein stores, the possibility of one tissue growing more rapidly or at the expense of another should be taken into consideration when interpreting

nitrogen balance data. With certain amino-acid imbalances, for example, liver protein may increase while some of the soft tissues of the body are losing protein, or vice versa.

PLATT: I strongly support that point of view; one of the things that the baby lives on when food is short is its glutei. Do you know what the soft tissues such as the liver contribute to the algebraic sum?

ALLISON: In protein depletion the liver proteins and the proteins of the gut are the most labile, often being reduced very rapidly. With certain amino-acid imbalances, however, the amount of liver protein may increase relative to the proteins of other tissues. I think that the most dramatic example of this imbalance in tissue proteins is produced by an abnormal tissue such as cancer. The cancer can be in strong positive balance, the liver may also be in positive balance, while the other soft tissues of the body may be in negative balance. The animal plus the tumour, however, will be in positive nitrogen balance.

We have put emphasis on the development of what might be called "lean body mass" in the animal, and on the maintenance of a proper balance in the enzyme systems of each tissue and between the various tissues, as our ultimate goal of protein nutrition.

DEAN: How significant, Dr. Holt, are the changes you found in serum protein concentrations? The level of serum albumin presumably reflects liver function, as it seems to be made only in the liver. This is a point of practical importance, because in the work I am doing on the utilization of different proteins, I am becoming more and more convinced that by following the changes in serum albumin, and in the activity of various blood enzymes, we may get real measures of the efficacy of the different proteins. I wonder whether you have any information on enzyme levels, showing rapid alterations with changes of diet?

HOLT: No, we have no data on enzyme levels. The changes in serum proteins are not altogether consistent, since not every child behaves in the same way. In threonine deficiency we got suggestive evidence of a fall in serum globulin, but the albumin level was maintained. We found no very definite change in the phenylalanine studies. Our impression from the two babies on lysine is that the albumin rather than the globulin was affected adversely.

DEAN: It is usually considered that the serum albumin or the serum proteins as a whole reflect the general level of depletion of the animal or of the human being. Is there good evidence of a close relation between the serum proteins and the rest of the proteins of the body? I cannot see any *a priori* reason why there should be.

SCRIMSHAW: We went into this a few years ago when we tried to find out why persons in under-developed areas, living on predominantly vegetable protein diets that we presumed were deficient, had serum protein values that were normal or even high. In Panama we found whole villages in which the average total serum protein was close to 8 or 9 g. per 100 ml. It appears from the world literature that predominantly vegetable protein diets *are* quite generally associated with normal or slightly high serum protein levels, and that the drop in serum proteins comes only with a relatively severe degree of protein deficiency in the diet.[41]

DEAN: Is the change in the albumin or in the globulins?

SCRIMSHAW: We have some electrophoretic patterns of sera from Central America with high protein values. These show that the increase is in both the albumin and the globulin fractions.

DEAN: When I feed a high-protein diet to children whom I presume to be protein-depleted, I get an increase not only in the serum albumin, which seems reasonable, but in the serum globulin, which seems unreasonable, because the starting level is already high.

ALLISON: If the total circulating albumin and globulins are measured, using plasma volumes, we find that the plasma albumin decreases in dogs with a reduction in liver protein and liver enzyme activity, while some plasma globulin fractions are unchanged. Repleting a depleted dog with wheat gluten resulted in a more rapid increase in plasma proteins than if the animal had been repleted with a better pattern of amino-acids for overall tissue protein synthesis.

Our general impression is that when a depleted animal is repleted, or during growth in the young animal, there can be an interchange between the tissues, leading to different rates of repletion or growth. Therefore the rate of repletion of plasma proteins does not always reflect the rate of repletion of other tissues.

COMPARISON WITH ADULT REQUIREMENTS

HOLT: It is interesting to compare our data on the amino-acid requirements of the baby with similar data on the adult. When this is done (see Table 6) it appears that the baby requires, in terms of mg. per kg. body weight, about ten times as much of each amino-acid as does the adult. In the case of phenylalanine the baby apparently needs considerably more than ten times as much.

TABLE 6

Comparison of minimal essential amino-acid requirements of adults and infants

Amino-acid	Overall minimal values (mg. per kg.)		Ratio of overall minimal values
	A. Adults	B. Infants	infants/adults
Isoleucine	10·0	90	9·0
Leucine	15·7	210	16·5
Lysine	11·4	105	9·7
Methionine			
cystine present ..	2·9	31	12·8
(cystine absent) ..	(15·7)		
Phenylalanine			
tyrosine present ..	3·6	90	26·1
(tyrosine absent) ..	(15·7)		
Threonine	5·4– 7·1	60	9·8
Tryptophan	2·9– 3·6	30	9·2
Valine	11·4–11·7	120	12·8

Note: A. Requirements determined by depletion studies on pure amino-acid diets. Data of Rose,[—] Leverton *et al.*,[12, 13] Jones *et al.*[11] B. Data from Table 5, column 9, p. 22.

Why does the baby require this great excess of amino-acids? Unlike the adult, he needs nitrogen for growth. He also has a higher basal metabolic rate, which gives rise to an increased maintenance requirement for nitrogen. In calculating the ratio of the infant to the adult requirement, as I have done in Table 6, I used Dr. Leverton's data on adults,[43, 44] rather than Dr. Rose's, because I did not have the data which Dr. Rose showed us on the requirement of phenylalanine in the presence of tyrosine. The ratio comes out at approximately 20 to 1.

The conclusion I reached was that growth is the chief cause of this difference. Perhaps somebody can clarify this matter or point out some fallacy which has escaped me.

GOPALAN: From the practical point of view it is very disturbing to hear tha the essential amino-acid requirement of infants per kg. body weight is as muc as ten times that of the adult. We have always been prepared to multiply th adult requirement by three in estimating the needs of the infant. It would appea from Dr. Holt's presentation that the position is much worse than that.

GYORGY: I would like to express a word of caution about the conclusio that the requirement of the growing infant for essential amino-acids is te times as high as that of adults, because the methods used by Dr. Rose on th one hand, and by Dr. Holt on the other, are very different. Dr. Rose used th nitrogen balance method, and Dr. Holt used chiefly the growth index. I am at loss, although I myself am a paediatrician, to compare the results obtained b these two methods and draw this conclusion of requirements in the ratio o 10 to 1.

MAYER: It is not so much that they are using different methods, but tha they are measuring different things.

GYORGY: That is true. In addition to that, I don't know how it is that D Holt has not been able to obtain a really zero balance or even negative balanc except in a very few instances. By contrast, in our own unpublished studie (in collaboration with Drs. Barness, Baker and Guilbert) it has been relativel easy to get zero balance in infants, just as Dr. Rose obtained it in adult We get it after about 6 or 9 days, not on an amino-acid mixture but on a mil formula. The figures obtained in our studies are identical with those reporte about forty years ago by Langstein and Edelstein in Germany,[45, 46] and, mor recently, by Dr. Kaye and his associates at the Children's Hospital i Philadelphia.[47] On a daily intake of 0·1 g. of nitrogen per kg. the nitroge balance may be zero, but usually it is definitely positive in young infants fe human milk or cow's milk formulae.

If we assume that 0·1 g. of nitrogen per kg. represents a minimum which wi support slightly positive nitrogen balance, comparable to the principle used b Dr. Rose for adults, we arrive at figures of *minimum* amino-acid requireme which are about one third to one fifth of the figures given by Dr. Holt. F instance, Dr. Holt gives the minimal requirement for lysine in terms of t monohydrochloride as 111 mg. per kg. (or approximately 96 mg. of free ba per kg.). Inasmuch as 0·1 g. of nitrogen is equivalent to about 50 ml. of huma milk, the minimal lysine requirement should correspond to the amount lysine present in 50 ml. of human milk, that is, 32 mg.[48] The figure for vali would be 27 mg. instead of 145 as given by Dr. Holt, and so forth.

We have to postulate, on teleological principles, that human milk is th physiological food. We may then further deduce that in human milk t

requirements for nitrogen (amino acids) and calories are in complete balance. The caloric requirement of a normal infant is covered by 150 ml. of human milk per kg. This would naturally multiply the figures given for lysine and valine by a factor of 3, and we will find the *optimum* for lysine as 96 mg. and for valine as 81 mg. per kg., still definitely below the figures of Dr. Holt. From a teleological point of view and from empirical knowledge, it is safe to state that with human milk an intake of 150 ml. per kg. should give the infant a very wide safety margin, with a satisfactory minimum below it.

MAYER: Do you define "minimum" in your infants as the amount of protein that maintains nitrogen balance but not growth?

GYORGY: It was not my intention to define the minimum; I don't think any definition can be made on an exact scientific basis. My object was to present data for the infant that could be compared with those Dr. Rose has given us for the adult.

MAYER: There is obviously a big difference between the situation in adults and in infants. In adults, if you get no change in weight, or if there is exact nitrogen balance, you can assume that the organism remains in an identical state; but in the child even though it is in nitrogen balance with a constant weight, there still may be a redistribution of protein taking place between organs, so I think that one has to be very careful.

GYORGY: I think that probably the same rearrangement may occur in adults as well.

HOLT: You say that 0·1 g. of nitrogen per kg. was adequate in the sense that it produced nitrogen retention. Translating that into terms of protein, it means 0·625 g. per kg. What was the weight performance, over a long period of time, of the child who received that much and only that much protein?

GYORGY: I cannot answer that question, because these were metabolic studies, just like Dr. Rose's. His experimental periods lasted 7 days and ours 9. During those 9 days there was no weight loss.* My comparison was only with the method that Dr. Rose used; we used a similar one for the purpose of measuring one special thing.

HOLT: Swanson's figures in Table 5, columns 2 and 3, were based on intakes of pooled analysed breast milk over periods of several months, so I think there is good reason to regard them as adequate. Swanson's child gained weight normally, whereas Kaye's cases apparently did not.

FREMONT-SMITH: That is a very important distinction.

GYORGY: Of course, but I emphasized that what I was talking about at the beginning was nitrogen balance.

MINIMUM AND OPTIMUM REQUIREMENTS

WATERLOW: It seems to me that there is no dispute here about facts, but a confusion about the use of the word "minimum". Dr. Gyorgy has used the word more or less to mean the amount needed for bare survival. Obviously if a child is in negative balance, it is going downhill and must soon die. The minimum for survival is that which just produces a positive nitrogen balance—

* In the experiments of Kaye *et al.*[illegible] 3 infants on an intake of 0·1 g. N per kg. per day gained on the average 15 g. body weight a day, which is close to the normal rate of weight gain at that age. However, the retention of electrolytes was greater, and that of nitrogen less, than could be accounted for if the gain in weight had been due to an increase in tissue mass. Therefore it seems possible that the gain was partly due to a deposition of fat, and partly to retention of electrolytes and water. (Editor.)

in fact, the same criterion as Dr. Rose has used. This is not, in my opinion, an academic figure: I think it is quite in keeping with intakes that we actually observe in under-developed countries. 0·1 g. nitrogen per kg. per day corresponds to about 3 g. of protein for a child weighing 5 kg., or the amount contained in 100 ml. of cow's milk. I think many babies in poor countries manage to survive on intakes of this order, or even less, but they do not gain weight. Dr. Holt uses the word "minimum" in quite a different sense, to mean minimum intake for normal weight-gain and what he calls "good" nitrogen retention.

CRUICKSHANK: It seems to me that in the growing child optimal and minimal must be approximately the same thing.

PLATT: We shouldn't think in terms of "minimal" and "optimal", but of "normal".

GYORGY: All right, I accept normal, and I would like to repeat that for human milk, or even cow's milk formulae adapted to human milk, the normal or *optimal* requirements are very close to the figures which Dr. Holt mentioned as *minimal* for a number of essential amino-acids, such as lysine, phenylalanine and threonine. We paediatricians are in a very fortunate position, because we are the only ones who can name the physiological food for the human beings we deal with. The physiological food for infants is human milk. We have data in the literature on many thousands of children, from which we get an average growth curve. Therefore one could say, with some justification, that an optimal growth curve in a single given infant should coincide with this average growth curve drawn from thousands of normal breast-fed infants. Everything below and everything above—I would like to emphasize, *or everything above*—is not optimal.

FREMONT-SMITH: You would allow a range?

GYORGY: Obviously there is a range; it depends, for instance, on the birth weight. But in general I think these conclusions are warranted.

SCRIMSHAW: Would you not also think of this in terms of change in length?

GYORGY: I am not too much concerned about that, because the length only increases from 50 to 70 centimetres in one year, whereas the weight trebles itself. Therefore the weight is a much better indication.

UTILIZATION OF PEPTIDES

GYORGY (continued): I should like to go on to the question whether the difference in outlook between Dr. Holt and myself—that what he calls minimal I call optimal—may not be due to the different methods used. In other words, is it permissible to compare protein with amino-acids? I am thinking of the possibility—at least for human milk and cow's milk—that peptides, which are very essential for the building up of new cells for growth, can be utilized much more easily than amino-acids. On such a vital question it is rash to conclude that we should have so much lysine, so much threonine, etc., without considering the possibility that the protein of cow's milk and of human milk might behave differently. I would like to express my doubts and to ask for caution in this respect.

ELVEHJEM: Both Dr. Rose and Dr. Holt used free amino-acids. Do you suggest, then, that peptides are more important in the infant—the growing individual—than in the adult?

GYORGY: I raise it only as a possibility, with special reference to the new, very interesting and careful investigations of Mellander in Uppsala, who has

shown[18, 50, 51] that milk protein when exposed to the enzymes of the gastro-intestinal tract is not broken down completely to amino-acids. Peptides are formed in considerable amounts with chelating capacity for calcium and iron. Such peptides are absorbed, and it is possible that, particularly in the growing child, they are used with greater ease than pure amino-acids.

TERROINE: There is a rather serious difficulty to be met by the hypothesis you have suggested to explain the superiority of milk. According to this hypothesis the digestion of milk proteins would give rise not only to free amino-acids but also to peptides. Now it does not seem to me that there is any reason why this should be advantageous. All theories of protein synthesis which have been put forward up to the present time suppose that this can only occur from free amino-acids. Is there any other process, apart from protein synthesis, in which peptides produced by the digestion of milk proteins and absorbed into the body could play a part?

GYORGY: In microbiology, more and more, we are aware of the fact that not only peptides of various kinds—and I refer to the strepogenin of Woolley[52, 53]—but also proteins act as growth-promoting agents. These are all non-essential growth-promoting substances, since bacteria, almost without exception, will grow in their absence, but they accelerate growth. Therefore, in analogy to our special problem in infants, we may have a better efficiency for growth from protein and peptides broken down from the protein, than from the final break-down products, amino-acids, which have been used by Dr. Rose and Dr. Holt.

COW'S MILK COMPARED WITH HUMAN MILK

DEAN: Do you expect to get the same results from 10 g. of human milk protein as from 10 g. of cow's milk protein?

GYORGY: My studies are not yet concluded. Cow's milk with a ratio of protein, lactose and fat adjusted to be the same as in human milk, appears to be slightly inferior to human milk, but only slightly, with regard to nitrogen retention.

DEAN: In the past we have always emphasized the superiority of human milk, so I find this very surprising.

GYORGY: I can also quote from the recent report of the Food and Nutrition Board of the National Research Council,[3] in which it is distinctly stated that "utilization of proteins from cow's milk is similar to that of the proteins from breast milk", and "there appears to be no convincing evidence that the breast-fed infant is lacking in protein". Therefore, if the utilization of cow's milk protein is similar to that of breast milk protein, cow's milk must be close to human milk, even with regard to the total protein requirement of the human infant. That is all I have in mind.

WATERLOW: Is the large amount of non-protein nitrogen in human milk, amounting in many specimens to about 40 per cent of the total nitrogen, available and utilized?

GYORGY: That, perhaps, would make the difference between cow's milk and human milk more significant. As you say, in human milk 20 to 40 per cent of the nitrogen is non-protein nitrogen, and a large proportion of this is urea. If we assume that urea is not utilized, then, of course, the utilization of human milk with its lower protein content is much better than that of cow's milk.

WATERLOW: So when you refer to so much nitrogen in human milk, you are talking of total nitrogen?

GYORGY: Yes. I said always 0·1 g. of nitrogen. I did not once use the factor 6·25.

ROSE: Urea is not utilized in animals unless there is a need for nitrogen. If you add labelled urea to an adequate casein diet, you get 100 per cent recovery in the urine. If, however, you have a nitrogen shortage, such as you can induce by feeding to the rat the minimal requirements of essential amino-acids, and then add urea to the food, the animal will immediately grow and utilize the urea nitrogen. If the urea is labelled with N^{15}, you can demonstrate the passage of the nitrogen to the amino-acids of the tissues.

DEAN: Does that apply to adult as well as young animals?

ROSE: I don't know. We have tried it only in growing animals.

WATERLOW: Rittenberg[34] showed some utilization of ammonium nitrogen in a human adult, but it was not as striking as in animals. An adult on a low protein diet excreted only 45 per cent of a dose of isotopic ammonium citrate in the first 24 hours, whereas a control subject on a high protein diet excreted 80 per cent. The corresponding figures in the rat were 30 per cent and 90 per cent.

Criteria for Requirements

ELVEHJEM: I am very glad to hear Professor Gyorgy equate minimum and optimum requirements in the growing infant. I, for one, am not afraid of minimal intakes and requirements. The minute we get below an adequate intake, we're in trouble; as soon as we are above the minimal requirement, we no longer have a deficiency. How much above the minimum we need to go, I don't know, but I think, oftentimes, we use two or three times the minimal requirements without realizing that such increases may be tremendous.

FREMONT-SMITH: Your minimum, though, is minimum-adequate, isn't it?

ELVEHJEM: Yes.

FREMONT-SMITH: That is important, because others have been using "minimum" in a different sense. You mean adequate with respect to what? To the growth curve, or some other criterion?

ELVEHJEM: In each case we must define adequacy under specific conditions. Dr. Rose used nitrogen balance in adult males, and he said that 0·15 g. of tryptophan was in most cases enough to establish nitrogen balance, although some individuals needed 0·25 g. Then he multiplied 0·25 by 2 to give a safe level. I suggest that this doubling may lead to excessively high figures for requirements.

APPEARANCE

ELVEHJEM (continued): Most of my experience has been with growth in animals, and I have obtained the impression that the animals on a minimal amino-acid intake often grow the best and show a certain sleekness—there is something about them that seems better to us than when they get twice or three times the requirement.

FREMONT-SMITH: Then you are almost saying that your minimum is your optimum?

ELVEHJEM: Yes, the minute you reach an adequate level, you have an optimum intake. For example, I might mention some results with rats on rice diets: 11 per cent of protein supplied by rice and a small amount of pork produced some of the nicest-looking animals that I have seen. Now 11 per

cent is considered a low level of protein, but these animals seem to perform better if they are right at the minimum intake. I think it is all very well to talk about safe levels, but if through worrying about safe levels we increase the intake two or three times, and end up with death from overnutrition before we ever encounter any of these limiting conditions, we have the wrong approach.

DEAN: You say the rats perform better? You mean they look better, in actual fact?

ELVEHJEM: I say there is something we can't describe by weight or by histological examination of the tissues. I think Dr. Catron will give the same impression from animal husbandry. Judges in animal husbandry will look at an animal and say, "There's something about this animal that is tops". You can often reach that situation at a minimal rather than a maximal level of intake.

DEAN: You seem to be still in the Dark Ages with your animals.

ELVEHJEM: I am not in the Dark Ages because I measure the intake of all our amino-acids, and I measure growth and carcass composition and enzyme changes in the tissues. But when all is said and done, some of us still work with the entire animal; if we did not get some challenge and some meaning out of such observations on the whole animal, then we would be studying only enzyme chemistry or histology or some other specialized aspect.

PLATT: I am entirely in agreement, and I think Dr. Dean would have to agree, that we really have no criteria for health. This is the problem that will have to be tackled in the future.

WATERLOW: I think everyone who deals with kwashiorkor will admit that you get a subjective clinical impression of improvement long before there is any objective change. Like Dr. Elvehjem I make enzyme measurements, but unfortunately so far they don't tell me anything that my eyes don't tell me first.

GYORGY: I would like to join the group who acclaim Dr. Elvehjem's statement.

TERROINE: I am very sorry that I cannot share in this acclamation; I think that we have made no progress at all, since we are still searching for a definition of "requirement". I have said several times that for adult human subjects there is only one sound baseline, namely the amount of a given protein that is necessary to maintain nitrogen balance. Is this criterion adequate? Drs. Mayer, Holt and Elvehjem have expressed their doubts. In that case I return once more to the question: what figure would they put forward, and on what basis? Dr. Elvehjem has just introduced a new idea, that of the good general appearance of the animal. This, however, is a matter of subjective impression only. It could not possibly, in my view, form the basis for any precise recommendations.

I agree with Dr. Elvehjem when he says that the desire to allow a margin of safety has resulted in figures for human requirements which are much too high. But I must ask once again what figure is proposed, and on what is it based, not only for the requirement of the adult, but also for that of the growing child? Dr. Elvehjem has spoken of a "minimum adequate" intake. What does he mean by this, and how can such a conception be justified?

KING: Dr. Elvehjem, I also must express a reservation on the concept you have put forward. Subjective appraisal is, perhaps, the most treacherous thing in the whole field of human nutrition, in part because we can never foresee the environment in which people may be placed. Growth is not an adequate criterion. For example, we have only very recently developed concepts of nutritive protection in maximum degree against dental caries. Apparently

there is a requirement for fluoride far above the level demonstrable by growth tests. Similarly, a new requirement has been indicated for vanadium well above the apparent requirement for growth. Again, if we take the case of Professor Sherman's long-term experiments, his famous Diet 16 animals grew at nearly the same rate as those receiving Diet 13, which contained more milk powder. Long-term or life-span performance records were needed to demonstrate the marked advantage of Diet 13.

In studies on vitamin C it has been shown that guinea pigs will grow normally by common standards with an intake of 1 mg. per day. They will be relatively sleek, fat animals when they are young, but as they get older they begin to lose muscle tone. They support reproduction poorly—only one or two litters—and then lose fertility and show resorption and other disturbances. An intake of almost 5 mg. per day or more is needed for optimum performance.

In one sense I agree that overall appearance and behaviour give a valuable index. An appraisal of total performance, whenever it can be done, may indicate the need for a safety factor against variables and stresses which have practical significance, but which are seldom measured by direct laboratory or clinical observation.

LEVELS SET TOO HIGH

ELVEHJEM: I want to repeat my original statement that I am not afraid o minimal intakes if they are adequate, and I think adequate and optimal ar the same thing. Dr. King mentioned fluorine for teeth. That is an excellen example: you don't want to double the intake of fluorine, just to make it safe you would get into difficulty if you did. Once the intake is adequate, you can' go much higher.

ROSE: Dr. Elvehjem has suggested that to allow a safety margin leads to levels which are much too high. If I could have a hundred, or, better, a thousand men on whom I had measured the tryptophan requirement, and if I knew tha every one of the hundred or thousand could be maintained on 0·25 g. a day then I wouldn't want to double that figure as a safety factor. I would fee convinced then that 0·25 g. is safe. On the other hand, when one must tak such a small sample of the human race for measurements of the kind I hav been trying to make, then I do feel that we must allow some margin of safety to take care of the possibility that somebody else, who has not served as a subject in these experiments, may have a still higher requirement. What tha margin ought to be, I don't know.

FREMONT-SMITH: Yes, a margin is needed to allow for individual variations

ROSE: Statistically, you can prove from the variations we get that twice th highest value will provide a margin that is adequate for everybody.

ELVEHJEM: Your safety programme takes care of one out of a hundred You are asking the other 99 individuals to metabolize an extra 0·2 g. o tryptophan every day and get rid of it, in order to take care of this one individual I maintain that that is a job for the clinicians, to find this one abnorma individual, and let us, in the average requirements that we recommend, tak care of the other 99.

Imbalance of Amino-acids

ELVEHJEM: This brings us to another related point which I think is ve important, and on which I would like to present some experimental data

In all our discussions so far we have talked about the ideal protein or the ideal combination of proteins. We should keep in mind that it is possible to have an imbalance of amino acids, both occurring naturally in proteins and resulting from amino-acid supplementation.

The beginning of our work on this goes back to a problem on pellagra which Dr. Aykroyd started, and which might be comparable to some of the problems encountered in kwashiorkor. People living on corn developed pellagra, but people living on rice were less susceptible to pellagra, and yet the corn diet supplied 15 mg. nicotinic acid daily while the rice diet supplied only 3 mg.[44]

TRYPTOPHAN AS A GROWTH FACTOR

ELVEHJEM (continued): Out of our study came the idea that tryptophan was a factor involved, because it was low in the corn proteins but higher in the other cereal proteins. By feeding a combination of a synthetic diet and certain cereals it was possible to correlate the rate of growth with the supply of tryptophan. Table 7 shows the growth response, tryptophan content and niacin content when 60 parts of the synthetic diet and 40 parts of the cereal were fed. The poorest growth was obtained with corn grits and yellow corn. Growth is obviously correlated more closely with the tryptophan content of the diet than with the niacin content.

TABLE 7

Effects of various cereals on growth of rats

Ration	Tryptophan (mg. per cent)	Niacin (per cent)	Growth (g. per week)
Synthetic 100	180	0·01	29
Synthetic 60 + corn grits 40	118	0·27	7
Synthetic 60 + yellow corn 40	128	0·92	13
Synthetic 60 + polished rice 40	138	0·56	31
Synthetic 60 + rolled oats 40	188	0·41	28
Synthetic 60 + white flour 40	151	0·40	29

Composition of synthetic ration

Sucrose ..	..	81·5
Casein ..	..	9·0
Methionine	..	0·3
Corn oil	..	5·0
Salts IV ..	..	4·0
Choline ..	..	0·13

Vitamins were included to provide, in mg. per kg. of ration: thiamine 5, riboflavin 5, niacin 9, calcium pantothenate 20, pyridoxine 2·5, folic acid 0·2, biotin 0·1, vitamin B_{12} 0·02, and ositol 100.

Further work showed that the addition of certain proteins low in tryptophan creased the requirement for tryptophan or niacin. The same effect was served when acid hydrolysates of proteins were used, since the hydrolysis estroyed the tryptophan.

The amino-acid which had the greatest effect on the tryptophan and niacin quirement was found to be threonine. If cysteine is added to a 9 per cent sein diet a slight growth increase is observed. However, if both cysteine and reonine are added growth is reduced to less than 4 g. per week. The addition niacin gives a growth response equivalent to at least 20 g. per week.

Further studies revealed that the livers from rats receiving 9 per cent casein diets were often fatty. The livers were enlarged and contained as much as 30 per cent of fat on a dry-weight basis. The addition of tryptophan increased growth but had no effect on the level of liver fat. However, a protein such as gelatin, which had been shown to increase the need for tryptophan, reduced the liver fat to a normal level of about 10 per cent. We thought that the amino-acid proline might be the active substance, since gelatin is rich in this amino-acid. Arginine was another possibility. Table 8 shows that neither of these added alone had any effect. The addition of 0·18 per cent threonine was found to be very effective in reducing the liver fat.

TABLE 8

Influence of amino-acids on growth and liver fat of rats fed 9 *per cent casein diets containing choline*

4-week feeding period

Diet	Growth (g. per week)	Liver fat (per cent dry wt.)
9 per cent casein-sucrose basal	17·7	30·1
Basal + 0·56 per cent L-arginine ..	13·8	26·2
Basal + 0·92 per cent L-proline	15·8	28·0
Basal + 0·1 per cent DL-tryptophan ..	20·9	31·4
Basal + 1·5 per cent glycine	16·4	18·3
Basal + 0·18 per cent threonine	9·7	12·4
Basal + 0·18 per cent threonine + 6 per cent gelatin	14·5	10·1

Threonine alone had no effect on growth, but when it was added together with tryptophan a good growth response was obtained, and the liver fat remained at a fairly normal level. The table also shows that a combination of threonine and gelatin is only slightly more effective in reducing liver fat than threonine alone. Threonine, therefore, has the greatest effect of all the amino-acids as far as the control of liver fat is concerned.

MAYER: So you were able to dissociate the agents that promoted growth from those that produced a decrease in liver fat?

ELVEHJEM: Yes. Threonine can affect the liver fat without having any effect on growth. By contrast, tryptophan causes a definite improvement in growth but has no effect whatsoever on liver fat.

SUPPLEMENTS OF INTACT PROTEINS

ELVEHJEM (continued): Table 9 shows what happens when we use intact proteins. Again the basal diet contained 9 per cent casein, and in this case we have shown liver fat on the fresh-weight basis in order to give some indication of the amount we might expect in ordinary liver. The addition of 3 per cent casein is sufficient to bring the liver fat down to a normal range, but not to give maximum growth. Six per cent of casein gives maximum growth and reduced the liver fat a little further. The addition of 3 per cent of gelatin has some effect on liver fat but very little effect on growth. Six per cent of gelatin is almost as effective as 3 per cent of casein as far as liver fat is concerned. Gelatin then, although considered a protein of poor quality, may be valuable in reducing

liver fat because it contains threonine and glycine. The last figure shows that whether we leave methionine out of the ration or add extra amounts it has no effect on liver fat. You remember that methionine has been known for a number of years as a lipotropic agent and it is lipotropic in a diet low in methionine. It has no effect in a diet which is adequate in choline but inadequate as far as other amino-acids are concerned. You might suggest that these effects are obtained only in the rapidly growing animal. However, you can produce typical fatty livers in adult rats by using 5 per cent of casein rather than 9 per cent, so this is not a condition confined to the young animal.

TABLE 9

Effect of proteins on growth and liver fat of rats fed 9 per cent casein diet

Supplement	Growth (g. per week)	Liver fat (per cent fresh wt.)
None	7·6	8·7
Casein 3 per cent	15·6	3·4
Casein 6 per cent	22·6	3·1
Gelatin 3 per cent	10·4	5·2
Gelatin 6 per cent	10·0	3·9
DL-methionine 0·3 per cent ..	14·9	8·4

DEAN: Has vitamin B_{12} any effect?

ELVEHJEM: None whatsoever.

DEAN: We have done experiments on rats in which we give the weanling offspring of vegetarian mothers the local Uganda diet; we can produce fatty livers, but we can protect the weanlings by giving some vitamin B_{12}.

ELVEHJEM: You are probably dealing with partial choline and methionine deficiency. B_{12} has a sparing effect on methionine and choline.

SUPPLEMENTS OF AMINO-ACIDS

ELVEHJEM (continued): The next table (Table 10) shows some results obtained when rice diets in rats are supplemented with amino-acids; I think this is a problem that is closely related to practical conditions.

TABLE 10

Effect of amino-acid supplements on growth and liver fat in rats fed rice diets

Diet and supplement	Growth (g. per week)	Liver fat (per cent dry wt.)
87 per cent rice	8·9	29·1
87 per cent rice + 0·2 per cent L-lysine + 0·2 per cent DL-threonine	22·0	28·9
87 per cent rice + 0·4 per cent L-lysine + 0·5 per cent DL-threonine	16·7	13·8
87 per cent rice + 0·2 per cent L-lysine + 0·5 per cent DL-threonine	23·3	22·0
87 per cent rice + 0·4 per cent L-lysine + 0·24 per cent DL-threonine	18·9	10·5
87 per cent rice + 0·2 per cent L-lysine + 0·24 per cent DL-threonine (+ 8 essential amino-acids)	25·5	27·8
87 per cent rice + 0·4 per cent L-lysine + 0·5 per cent DL-threonine (+ 8 essential amino-acids)	30·6	14·9

1. *Lysine and Threonine*

Rice was used as the sole source of protein. On the basal rice diet growth is poor and the liver fat is high, as you would expect from the previous results in Table 9. If we add the two most limiting amino-acids, lysine and threonine, at the level of 0·2 per cent, there is an improvement in growth, but the liver fat remains high. This is quite in contrast to the results obtained when a good quality protein such as fibrin or pork muscle is added. At these levels, therefore, lysine and threonine are sufficient to improve growth but they have no effect on liver fat. In fact, you may be doing more harm than good by supplementing the rice diets with these amino-acids. However, if you double the level of lysine to 0·4 per cent and of threonine to 0·5 per cent, liver fat is significantly reduced, but now we obtain an inhibition in growth. The level is now so high that we are producing an imbalance of some kind. If eight essential amino-acids are added to the high level of lysine and threonine the liver fat is in the normal range, and excellent growth is obtained. We have recently shown that of the essential amino-acids, valine plays an important part in counteracting the growth inhibition.

2. *Leucine and Isoleucine*

Table 11 shows another type of imbalance. In this case leucine has a very definite growth-retarding effect when added at a level of 3 per cent to a low protein diet. If the same level is added to a diet containing 18 per cent of casein

TABLE 11

Retarding effect on growth of excess leucine

Diet	Wt. gain (g.) 1st week	2nd week
9 per cent casein	15	18
9 per cent casein + 3·0 per cent L-leucine	−2	4
18 per cent casein	32	42
18 per cent casein + 3·0 per cent L-leucine	28	43

no inhibition appears. There must be something in the extra 9 per cent of casein which will counteract this effect of leucine. Table 12 shows that isoleucine is the amino-acid which has this effect.

TABLE 12

Effect of isoleucine in counteracting excess leucine

Addition to 9 per cent casein ration (per cent)		Wt. gain (g.)	
L-leucine	DL-isoleucine	1st week	2nd week
—	—	15	18
1·5	—	0·5	9
1·5	0·4	9	18
3·0	—	−2	4
3·0	0·8	10	13

An addition of 0·4 per cent of isoleucine will counteract 1·5 per cent of leucine and 0·8 per cent of isoleucine will counteract 3 per cent of leucine. It is important to point out that the isoleucine does not have the same effect as 9 per cent of casein. Recent work has shown that valine is one of the amino-acids which appears to be quite effective. Interesting relationships appear to exist between leucine, isoleucine and valine. The inhibitory effect of leucine is of considerable significance, since the corn protein zein contains a lot of leucine, 23 per cent. Dr. Platt has described a diet in which the leucine intake was about eleven times the isoleucine intake. Such relationships might easily be encountered with corn diets. These are not only high in leucine, but the isoleucine which is present in zein appears to be unavailable. Therefore it is a problem of practical importance to find a way of overcoming this leucine-isoleucine imbalance.

We have made another very interesting observation in some studies on the effect of fertilization on the protein content of corn. As the protein content increases during fertilization, most of the amino-acids increase as well. However, lysine actually decreases, and leucine shows a very significant increase; so that by fertilizing the corn you are increasing the level of an amino-acid which may produce imbalance.

I recognize that these studies are all on animals and may or may not be directly relevant to problems in man, but, in calculating amino-acid intakes, especially from vegetable proteins, I think it is well to keep these relationships in mind.

GYORGY: Were these experiments done with paired feeding? Was the intake identical in all groups?

ELVEHJEM: No, the animals were fed *ad libitum*.

GYORGY: Is it not possible that with 3 per cent leucine the appetite of the rats for some reason was reduced?

ELVEHJEM: I rather doubt it. I don't know why they shouldn't like leucine and should like isoleucine. Usually you find an increased appetite with a decrease in free amino-acids, but here you are adding another amino-acid on top of the leucine.

KING: What kind of protein would most efficiently bring this leucine-isoleucine ratio into a more nearly ideal balance?

ELVEHJEM: I think any animal protein will work very well.

KING: Have you identified any plant proteins that would help to do it?

ELVEHJEM: No, we have not tried any plant proteins. But, of course, it is perfectly obvious that corn is the worst offender; almost any other vegetable protein would tend to counteract the high level of leucine in corn, so that a combination of vegetable proteins would be much better than a single corn protein.

AYKROYD: I spent a rather large part of my life trying to supplement rice with various kinds of foods in animal experiments, and I am struck by Dr. Elvehjem's experiments showing the remarkable growth-enhancing effect which certain amino-acids produce when added to a rice diet. I think these very striking results are most relevant to the whole problem under discussion here.

AVAILABILITY OF AMINO-ACIDS

ELVEHJEM: The next problem, if we are going to think in terms of amino-acids, is that of availability, and on this we have relatively few data. Special

emphasis has been given to the availability of lysine and, perhaps, of a few other amino-acids, but some aspects of the problem such as the unavailability of isoleucine in corn, have not been recognized at all; so I think we need a great deal more work on the availability of amino-acids. Some of that work has been done by measuring the so-called biological value of proteins, but I think more should be done on the individual amino-acid.

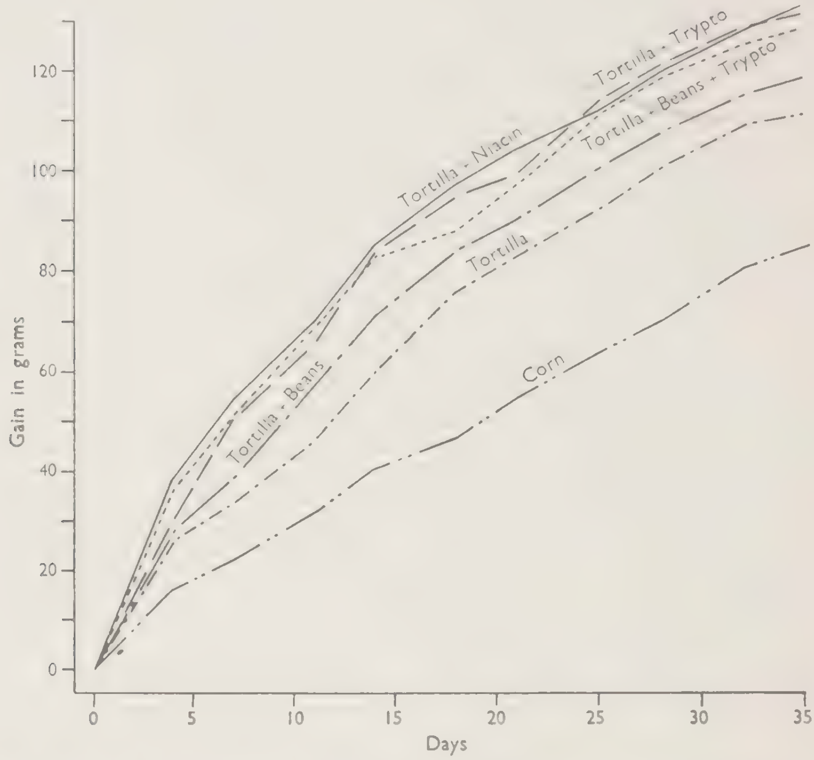

FIG. 5. The effect of the addition of beans, niacin, and tryptophan to corn and tortilla diets fed to niacin-depleted rats.

SCRIMSHAW: I think some findings that we reported recently are pertinent.[56] Rats were fed on a basal ration lacking in niacin and low in tryptophan. When the raw corn in this ration was replaced by lime-treated corn in the form of tortillas, growth was significantly improved (Figure 5). When beans were added to the latter ration, in the proportion consumed in human diets of the region, growth was further improved, although the difference fell short of statistical significance at the 5 per cent level. When either niacin or tryptophan was added, maximal growth was obtained. Obviously the lime treatment of corn in some way improved its nutritive value for these rats. It appears most probable that the amino-acid balance was improved, either by partial destruction of certain amino-acids, or by making others more available.

Amino-acid Deficiencies in Kwashiorkor

HOLT: It seems to me that if we look ahead, we shall find that the concept outlined by Dr. Elvehjem, of amino-acid deficiency and imbalance, may help us to understand some of the puzzling features of the kwashiorkor syndrome. I think the picture that we call kwashiorkor may represent to a large extent

specific amino-acid deficiencies rather than protein malnutrition *per se*. It is my personal belief, with which others here may differ, that some of the differences observed in the clinical picture in different parts of the world are due to the fact that the amino-acid intakes are different in different parts of the world. Even with our present limited knowledge it is possible to ascribe some of the symptoms to such a basis; the hair changes, for example, may well be due to a deficiency of aromatic amino-acids. We have no observations on the treatment of kwashiorkor with aromatic amino-acids, but we have observed that a similar dyspigmentation seen in phenylketonuria will respond to the administration of tyrosine alone. I am therefore inclined to ascribe the hair changes of kwashiorkor to a specific amino-acid deficiency.

ABNORMAL METABOLISM OF TRYPTOPHAN

HOLT (continued): We also have chemical evidence for abnormalities of amino-acid metabolism in kwashiorkor. Table 13 illustrates an abnormality of tryptophan metabolism. The data were from a patient in Dr. Gomez' clinic in

TABLE 13

Nitrogen and tryptophan metabolism in a child aged 1 *year with kwashiorkor*

Time	Diet	Nitrogen balance data					N-methylnicotinamide in urine (mg.) after 1 g. L-tryptophan given intravenously			
		Intake (g.)	Urine (g.)	Stools (g.)	N absorbed (g.)	N absorbed (%)	1st day	2nd day	3rd day	4th day
On admission	Tortilla and beans	7·33	3·35	4·96	2·37	32	0·81	1·76	5·18	5·90
1 week later	Milk added	7·68	2·81	3·04	4·64	60	0·55	0·78	0·75	0·81
2½ months later	Milk	13·32	11·51	1·95	11·37	85	1·97	3·06	1·98	1·53

Mexico City. Some of the chemical measurements were made by members of his staff and some were made in our laboratory in New York. Three sets of observations were made; immediately after admission (on a diet of tortilla and beans), 1 week later after the addition of skimmed milk and some 2 months later on a liberal diet which provided milk in quantity. The absorption of nitrogen was poor initially (only 32 per cent of the intake), but improved with recovery; this has been observed by other workers. The nitrogen retention was also poor to begin with and much of the absorbed N was lost in the urine. The nitrogen retention, too, improved after one week. At each of these three periods we carried out a tryptophan load test, 1 g. of L-tryptophan being given intravenously and the urine being followed for several days for N-methyl nicotinamide. It may be seen that on admission the administration of tryptophan was followed by a striking rise in N-methyl nicotinamide excretion. This indicated that there was no lack of vitamin B_6 or of the enzyme kynureninase. Somewhat to our surprise, the findings one week later were quite different; there was a gain in weight, nitrogen retention had improved and the administration of tryptophan now caused no increased excretion of N-methyl nicotinamide.

Later during recovery the N-methyl nicotinamide response to tryptophan reappears. Our interpretation of these findings is as follows: initially it would appear that enzymes necessary for tissue synthesis were deficient and that little nitrogen could be used for this purpose; an excess of tryptophan was hence available for degradation to N-methyl nicotinamide. One week later, with the restoration of synthetic processes and the consequently greater demand for tryptophan, no surplus remained for degradation to N-methyl nicotinamide. As the demands for growth became satisfied the excretion of N-methyl nicotinamide reappeared.

The observations made in this patient are not unique. We have observed similar changes in several other subjects. We have studied the excretion of free tryptophan in the urine by means of paper chromatograms. Under ordinary circumstances tryptophan cannot be demonstrated by this technique. After a load test, however, we have been able to demonstrate it consistently on admission, but only irregularly thereafter. There is thus a rough parallelism between the excretion of free tryptophan and an increased N-methyl nicotinamide excretion. Tryptophan spills out when it cannot be readily utilized for tissue synthesis.

DEAN: Did you investigate pyridone?

HOLT: No.

DARBY: Did you measure the excretion of vitamin B_6?

HOLT: Not in this particular patient, but in a number of others we obtained no evidence of B_6 deficiency from observations on the urinary excretion of the vitamin.

EXCRETION OF FREE AMINO-ACIDS

HOLT (continued): We made some further studies on a typical case of kwashiorkor observed in New York City. The urine of this patient was studied for total free amino-aciduria, and also for specific amino-acids by the chromatographic technique of Moore and Stein with a Dowex-50 column. Four observations were made: the first on admission, the second during the development of the "recovery syndrome of Gomez", and the third and fourth during its defervescence.

TABLE 14

Excretion of free amino-acids by a child aged 5½ years with kwashiorkor

Date	4 Nov., 1954	19 Jan., 1955	14 Mar., 1955	22 Apr., 1955
Clinical condition	Chronic malnutrition	Early recovery	Post-recovery	Post-recovery
Weight (kg.)	11·9	15·0	16·2	16·7
Urine volume (c.c. per day)	45	315	350	263
Total N in urine (g. per day)	0·45	3·23	3·39	3·02
α-amino N in urine (mg. per day)	14·7	71·5	84·3	45·3
$\frac{\alpha\text{-amino N}}{\text{Total N}}$ (per cent)	3·27	2·21	2·48	1·50
Creatinine (mg. per day)	68	203	222	206
$\frac{\alpha\text{-amino N}}{\text{Creatinine}}$	0·216	0·351	0·380	0·22

Table 14 shows the output of free amino-acids. This requires definition, since amino-acid excretion may be expressed in several ways—as total daily excretion, as a function of weight or body surface, as a function of total nitrogen excretion, etc. We have chosen to express it in terms of daily creatinine output, although other indices are also shown in the table. You will note that the ratio of free amino-acid to creatinine rises from 0·21 to 0·35 with the development of the "recovery syndrome", and that it subsequently falls to 0·22. The liver was still enlarging at the time of the second observation, but declining at the third and fourth; some hepatomegaly was, however, still present at the last observation. It appears that amino-aciduria is characteristic of the "recovery syndrome", but not of early kwashiorkor. We have observed increased amino-aciduria in only 1 case initially out of 5 patients studied.

FREMONT-SMITH: Could the amino-aciduria be accounted for by the increase in volume of urine?

HOLT: No.

GYORGY: How much protein did you give that child?

TABLE 15

Excretion of individual free amino-acids in normal subjects and three patients with kwashiorkor expressed in mg. per g. creatinine

(One patient is the one for whom figures are given in Table 14 studied over the same four periods.)

Amino-acid	Normal		Kwashiorkor patients					
	Average of 3 infants 1–6 months	Average of 2 children 10 years	Cruz (5½ years) I	II	III	IV	Morraquin (3 years)	Buendia (2 years)
Taurine*	trace	130	0	28·8	7·3	trace	0	17·0
Threonine	80	26	10·1	61·6	47·0	32·1	trace	53·0
Serine (+ asparagine and glutamine)	170	64	86·5	182·0	199·0	134·0	14·7	264·0
Glutamic acid†	95	13	49·1	36·5	38·0	19·0	28·6	36·0
Glycine	352	120	366·0	621·0	738·0	297·0	203·0	348·0
Alanine	150	34	38·2	147·0	161·0	106·0	63·8	165·0
Cystine	37	12	16·9	27·6	12·6	20·1	0	0
Valine	§	9	15·6	14·6	22·1	17·1	11·8	trace
Methionine	10	trace	24·2	23·4	32·0	trace	0	trace
Isoleucine	17	14	57·2	28·6	41·0	28·6	25·6	25·0
Leucine	46	15	16·2	14·8	25·0	44·7	0	19·0
Tyrosine	79	34	32·6	23·7	42·4	25·4	56·2	32·0
Phenylalanine	54	15	14·4	108·4	113·0	74·3	76·7	79·0
3-methyl histidine	44	58	44·0	46·6	40·0	?	28·4	not studied
Histidine	378	252	254·0	296·2	352·0	244·0	148·5	
		92						
Lysine‡ (+ 1-methyl histidine)	95	278	75·9	55·2	75·0	62·4	36·6	

* Taurine excretion is conspicuous in first days of life, but virtually absent in older infants. The exact age at which it tends to reappear has not been ascertained.

† These figures are not precise since the glutamic acid increases during storage. This is probably due in large part to the conversion of glutamine to glutamic acid.

‡ The lysine peak in the chromatogram includes 1-methyl histidine which comes down at the same time. Simultaneous studies on paper have shown that in young infants most of this peak is due to lysine, whereas in older children and adults it is mostly l-methyl histidine.

§ Valine is difficult to measure accurately in infants because of overlapping with unknown peak which is prominent at this time.

HOLT: She was fed milk, and a mixed ward diet. She was not on a balance study at this time.

GYORGY: Protein intake was not excessively high?

HOLT: No. Table 15 shows the excretion of individual free amino-acids in this same patient and 2 others with kwashiorkor, expressed also in terms of creatinine output, and compared with normal values. I would call attention to three specific changes: (1) a decreased initial output of threonine, (2) a higher excretion of isoleucine at first compared with that of leucine, due to increased excretion of isoleucine, and (3) a high ratio of phenylalanine to tyrosine excretion during recovery, caused by excess excretion of phenylalanine. The low threonine excretion is not always found, and, moreover, was found here only initially. These findings were also present in 2 other cases from Mexico studied only on admission.

I am not prepared to interpret these findings, though it is tempting to relate the increased excretion of phenylalanine to the dyspigmentation of the hair. The observations on the leucines also make one think of the imbalance between them of which Dr. Elvehjem spoke.

These, of course, are only the most preliminary findings. I don't think that as such they prove anything, but I present them in the hope that other people will be stimulated to study particular amino-acids further, in blood as well as in urine. It seems to me that the key to the kwashiorkor syndrome may come from studies of specific amino-acid metabolism.

HANSEN: In a few cases we have measured amino-acid excretion by the Moore and Stein technique. It was interesting to find, on comparing our figures with Dr. Holt's, that in one case we seem to have the same sort of changes that he has found in the ratio of isoleucine to leucine, and of phenylalanine to tyrosine.

WATERLOW: Surely you can't argue that these changes in ratio are necessarily the result of deficiencies in the diet? They might equally well result from exchange of amino-acids between proteins of different composition in different sites and tissues of the body.

HOLT: Yes. There are other possibilities too. They might represent alterations in kidney function. My only point was that we need further studies to find out what they do mean.

WATERLOW: One way of testing your hypothesis would be a therapeutic trial with single amino-acids, although such trials are extremely difficult to carry out, as I learnt when I was testing the effect of methionine.[57]

Therapeutic Tests in Kwashiorkor

HANSEN: We have done some work in South Africa which forms the necessary background for tests of that kind. The results have been published,[58] and therefore need only be summarized briefly here.

TREATMENT WITH SKIMMED MILK

HANSEN (continued): The basis of the investigation was this question: if skimmed milk cures kwashiorkor, what is the curative factor? Is it the proteins, the vitamins, the minerals, or the fat?

Four diets were used:

(i) skimmed milk with a vitamin supplement;
(ii) skimmed milk alone;
(iii) so-called vitamin-free casein, with added minerals, dextrose and vitamins;
(iv) casein, minerals and dextrose without vitamins.

The first problem was to choose the criteria of cure. The experimental period was to be short; therefore we coined the term "initiation of cure". This meant that within 21 days the serum albumin should have risen; the child should have lost its oedema and regained its appetite; apathy should have gone, and the skin lesions be healing.

A further difficulty was that, although all cases were given penicillin and sulphadiazine, it was sometimes necessary in the patient's interest to use other antibiotics as well, or to give transfusions of blood or plasma. Therefore for the purpose of this trial all such cases that needed extra treatment have been classed as failures, although many of them did in fact show initiation of cure within 21 days. Table 16 summarizes the results. What this table shows is the ability of a given regimen to initiate cure in 21 days without any other treatment except penicillin and sulphadiazine. There was no statistically significant difference in the cure-rate on the different diets.

TABLE 16

Treatment of kwashiorkor with skimmed milk and casein mixtures

Formula	No. of cases	Cured (per cent)	Failed (per cent)	Causes of failure (nos. of cases)		
				Extra treatment	Death within 48 hr.	Death later
Skimmed milk + vitamins	28	61	39	7	1	3
Skimmed milk, no vitamins	33	42	58	9	4	6
Casein + vitamins ..	44	59	41	8	2	8
Casein, no vitamins ..	30	50	50	12	0	3
Synthetic (amino-acids) ..	9	56	44	3	1	0

GYORGY: The table shows that you gave transfusions to a larger percentage of the children treated with vitamin-free casein, compared with the group receiving skimmed milk plus vitamins. I concede that you have listed these as failures. However, my doubts go deeper. These children might have been saved from death *because* of the transfusions. This might indicate that skimmed milk, especially with vitamins, is superior to vitamin-free casein alone.

SCRIMSHAW: In fact, whenever you have a child who is not responding, and you are afraid of losing him, you give a transfusion?

HANSEN: Yes; but the 21-day cure rate, which is what we are interested in, is still not significantly different with the two treatments. Maybe a difference would show up with a larger series.

PLATT: Why do some of the cases not respond?

HANSEN: I think it is partly because some of them have very severe infections, with very bad diarrhoea. A number of the deaths have been unexplained. They die very suddenly.

GOPALAN: Have you taken account of the time taken for good effects to be observed—for example, the time for oedema to disappear?

HANSEN: The time has varied between 12 days and 21 days for initiation of cure. We have not gone beyond 21 days. If a subject has not shown response within that time, the treatment has been regarded as a failure. All the cases that responded lost their oedema between 7 days and 15 days at the most.

GOPALAN: I think it is extremely important to decide the criteria for assessin the effect of different lines of treatment, because the therapeutic tool gives som guidance in the planning of a preventive campaign. It seems to me that th criteria employed here are not quite valid.

SCRIMSHAW: Well, they are different. Dr. Hansen is really taking the dis appearance of acute symptoms as the criterion, without following the childre until they reach a period of reasonable growth. In Central America, and, assume, in your country also, we do not consider children cured until they hav entered a period of steady growth.

TREATMENT WITH AMINO-ACID MIXTURES

HANSEN: We have also treated nine patients with a synthetic amino-aci formula. This contained 18 amino-acids, which together supplied 59 per cen of the calories, together with vitamins, dextrose, and minerals. The results ar also shown in the table. The cure-rate is much the same as with the other diets

Just before I left Cape Town we had begun trials with a sixth formula whic contained only 11 amino-acids—the 8 essentials plus arginine, histidine an tyrosine in the lowest quantity calculated to be adequate for nitrogen balance These together supplied 16 per cent of the calories. Four patients who hav been tested seemed to be responding quite well, but at the end of 14 days w got the clinical impression that they did not look quite so happy as the others perhaps because of the diarrhoea produced by the amino-acid mixture.

CRUICKSHANK: Have you considered making another trial of ventriculin in view of the Gillmans' results?[59]

HANSEN: We haven't thought of it.

PLATT: Was the synthetic diet equivalent in amino-acid content to the casei diet?

HANSEN: Yes; in the first synthetic amino-acid mixture there was a highe percentage of nitrogen than in the casein formula. We had the same scheme fo building up intake in all of them. The total intake aimed at was $2\frac{1}{2}$ oz. per lb body weight.

PLATT: Did you make any deduction on the equivalence of amino-acids casein and whole milk protein, as a by-product of your work, that might be useful to us in this discussion?

HANSEN: I think we may do so later. I wouldn't like to draw any conclusion yet. All I will say is that the synthetic amino-acid mixture seems to give good results.

MAYER: In view of Dr. Rose's results, wouldn't you expect to need more calories with the amino-acids than with the proteins?

HANSEN: I don't know. The formulae all had about the same caloric content. The calorie intake varied between 600 and 900 calories per day when intake was maximal. This was in children ranging in age from 12 months to 3 years, and in weight from 12 to 25 lb.

SCRIMSHAW: Those of us working in the field know the difficulty of assembling so many cases and of making these therapeutic trials, and the results are very valuable.

CRITERIA OF CURE

GOPALAN: Before we draw any conclusions from these very interesting observations, we should examine more closely the validity of some of the

criteria that were employed. I think that children who develop kwashiorkor have been subsisting on such poor diets before they come to the hospital that it is possible to show striking improvement with any reasonably good type of therapeutic regimen. For example, we tried to work with skimmed milk in one group and with a protein which is known to be decidedly inferior to skimmed milk—namely Bengal gram—in another; by clinical criteria we would say that the two lines of treatments were equally effective. Would we then be justified in equating skimmed milk with Bengal gram? Actually, body composition studies in animals revealed significant differences in the ability of these two sources of protein to promote tissue regeneration in depleted animals. I should be inclined to say that the clinical criteria are too crude to show up differences in response to the two proteins. Therefore I think we must be very careful. We may have to use a whole battery of clinical criteria, like the time taken for minimal weight to be reached, the time taken for the body weight to increase by 1 lb. after attainment of minimal weight, etc. With such a multiplicity of criteria we may be able to make a more precise assessment of therapeutic response.

SCRIMSHAW: I both agree and disagree with you. If you can get an initial response with vegetable protein fed at 1 g. per kg. equal to that with the same amount of skimmed milk protein, it is very important. Then we can go on and refine the experiment, and find out what we need to produce satisfactory responses by other criteria.

MAYER: Is it not true that the purpose of the experiment was not to compare various types of proteins, but to find out what, in skimmed milk, was the effective agent?

HANSEN: That was the primary purpose.

GOPALAN: Even that conclusion would not be justified on the basis of these observations. That is my contention.

ROSE: I think it is astounding that Dr. Hansen has succeeded so well in administering amino-acid diets to sick children. Such diets are difficult for an adult to consume, even when he has an interest in the outcome of the experiment. For these sick children to eat the diets so successfully is, I think, really remarkable. We owe Dr. Hansen a vote of thanks. Furthermore, it does not follow that the therapeutic use of amino-acids would necessitate as high a calorie intake as would be needed for nitrogen balance.

KING: So far we have been following up the point thrown out by Dr. Aykroyd towards the end of his introductory remarks, when he said that the time was perhaps ripe to consider protein requirements in terms of amino-acids. We might call this the biochemical approach, and in pursuing it we have accepted more or less implicitly two criteria of an adequate protein intake—normal growth in infants, and the maintenance of nitrogen equilibrium in adults. I think we should now approach the problem from a more physiological angle, and consider the validity of these criteria, particularly the second—that of nitrogen balance. I believe Dr. Allison has data which would help us here.

Protein Depletion in Dogs

NITROGEN BALANCE AS A CRITERION OF ADEQUATE INTAKE

ALLISON: I have tried to pick out observations that may illustrate some of the problems which we have been discussing, and some of the types of research into which we might go to help solve these problems.

Considering first nitrogen balance, we have been trying to determine the amount of protein that is essential for maintenance of nitrogen equilibrium in the so-called normal adult dog.[60] The results varied from 0·15 to 0·2 g. of nitrogen per day per kg. of body weight.

The data presented in Figure 6 represent essentially short-term experiments. The point I want to emphasize is that the intake needed to produce balance depends not only on the type of protein fed, but also on the state of the dog's protein stores. Curves A and C are from dogs fed a good protein such as whole egg or milk; B and D illustrate the type of data obtained with wheat gluten, a protein deficient in lysine, methionine and cystine, and perhaps low in tryptophan. A and B are from depleted dogs. C and D from animals with full protein stores. Curve A shows that in the depleted state nitrogen equilibrium can be maintained on only about 1 g. of egg nitrogen per sq. m. of body surface

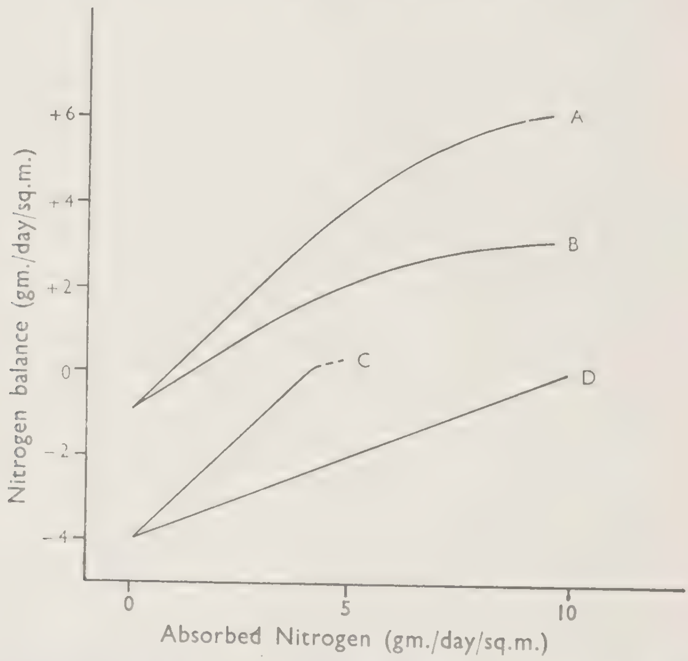

FIG. 6. Nitrogen balance vs. absorbed nitrogen in a protein-depleted dog fed whole-egg protein (A), in a protein-depleted dog fed wheat gluten (B), in a normal dog fed whole egg (C), and in a normal dog fed wheat gluten (D). (From the original of Figure 3, J. B. Allison, *Fed. Proc.*, **10**, 677, by kind permission of the publishers.)

per day, whereas in the dog with full protein stores (C), about 4 times as much is necessary. Curves B and D show the same thing: less nitrogen is needed to maintain equilibrium in the depleted animal. In general, the further an animal can be placed in positive balance by a given dietary protein intake, the greater the degree of depletion. If one compares the curves from dogs on egg protein with those on gluten, it is clear that a protein of low nutritive value will not produce as great a positive balance as one with a higher value, no matter how much of the poorer protein is fed.

WATERLOW: Would it be correct to say that the greater the degree of depletion, the lower the level on which you can maintain balance, so that that level could almost be used as a test?

ALLISON: Yes. Under these experimental conditions the amount of nitrogen necessary to maintain equilibrium, or even better, the degree of positive balance that you can produce with a certain intake, is a good measure of the degree of depletion.

By feeding a low nitrogen intake it is possible to maintain a dog in a depleted state for quite a long time—I don't know exactly how long. The depleted animal develops symptoms of protein deficiency, similar to those described in kwashiorkor. The data in Figure 7 illustrate some of the changes that take place in the circulating fluids of protein-depleted dogs.[61] The total circulating alpha and beta globulins are not reduced under the conditions of protein

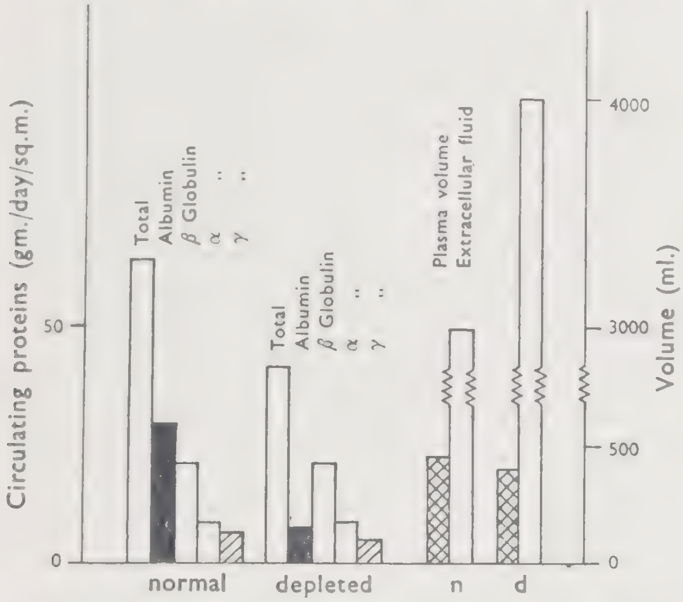

FIG. 7. The total circulating proteins, plasma albumin, alpha, beta and gamma globulins, before and after protein depletion. The figure also shows the plasma volume and extracellular fluid in normal dogs (n) and depleted dogs (d).

depletion in these animals. The main decrease is in the albumin, often accompanied by a decrease in gamma globulin. The plasma volume is reduced, and the reduction may be so marked that even though the total circulating protein is decreased, there is little if any fall in protein *concentration*. Measurement of total circulating protein or of the albumin globulin ratio is almost essential to detect this type of depletion.[61, 62]

There is an increase in extracellular fluid, measured as sodium thiocyanate space. Oedema may not be obvious clinically, or it may be just detectable around the toes.

GOPALAN: It is an interesting point that there is a diminution in plasma volume. We have made some observations in India on this. Much of the diminution in absolute amounts of serum constituents can be masked by the concomitant fall in plasma volume. This is a point to be remembered when we try to interpret the significance of serum albumin concentration, at any rate in the early stages of protein malnutrition.

SENECAL: How long does it take for these changes to develop?

ALLISON: I think a normal dog fed a protein-free diet would begin to show these changes within a week, and at the end of three weeks to a month you would probably have all the symptoms of severe depletion.

GOPALAN: Have you tried to replete the animals and reverse these changes?

ALLISON: Yes, we have repleted all these animals and reversed the changes, and they all can come back to normal. As far as we can tell, there is no irreversible damage, unless they are kept in this depleted state too long.

GOPALAN: During repletion did you observe the same phenomenon which we find in children recovering from protein deficiency?—that is, in spite of a positive nitrogen balance over a period of several weeks, the body weight remains stationary?

ALLISON: Very often the weight does not change for a time, even though the animal is being depleted or repleted. Of course, during repletion the extracellular fluid decreases.

WATERLOW: Are the dogs in nitrogen equilibrium at the time when these changes occur?

ELVEHJEM: They must have been in negative balance at some point.

ALLISON: Yes. In order to deplete the protein stores, negative nitrogen balance must be produced. A normal dog needs, let's say, 0·2 g. of nitrogen per day per kg. to maintain equilibrium. If the nitrogen intake is reduced to 0·05 g. per kg. per day, the dog would go into negative balance, and then gradually, as the protein stores are depleted, the animal would drift into nitrogen equilibrium on this same low nitrogen intake.

DARBY: Is this not a principle that applies to measurement of balance with a number of elements? I recall that Dr. H. H. Mitchell a good many years ago demonstrated that if one starts at a high level of calcium intake, it requires a high level to maintain balance. If, then, you cut this to half, calcium balance is negative for a while, but equilibrium is then re-established at the lower level, and you can keep going down and down. From the standpoint of arriving at requirements from balance studies, it is very important for us to bear this in mind.

SCRIMSHAW: This is what Hegsted found in studies in Peru, where a very low calcium intake sufficed to maintain subjects in calcium balance.[63] But, of course, it also raises the question as to what that calcium balance meant.

ALLISON: That is the main point, that the maintenance of nitrogen balance has to be interpreted in terms of other factors.

PLATT: The important thing is that the existence of nitrogen balance is no real criterion of the subject's state of protein nutrition.

WATERLOW: That is very true. The lowest level of nitrogen balance is not an adequate criterion, nor is it particularly logical simply to double that level. We surely can't suppose that animals or people with depleted protein stores are equivalent, in terms of health and function, to those with normal stores.

TRANSFER OF NITROGEN WITHIN THE BODY TISSUES

WATERLOW (continued): In the present state of knowledge it is difficult to find concrete experimental evidence on this point. But I think we are gradually coming to realize the extent and rapidity of the shifts of protein and amino-acids that may occur between different organs within the body. We might almost think in terms of a kind of internal circulation of nitrogen. I would like to draw your attention to the experiments done in Denmark on human subjects by Olesen, Heilskov and Schonheyder.[64] These are an extension of the early work of Schoenheimer and Rittenberg. They gave isotopic glycine, and followed the excretion of labelled nitrogen for 30 days. From their results they concluded that there is a pool into which dietary nitrogen comes, and from this pool nitrogen is taken up on the one hand into rapidly metabolizing tissues, such as liver and pancreas, on the other by slowly metabolizing tissues. This is not a new conception: the important point is the *magnitude* of the rates of exchange between the tissue pools. On a dietary intake of 10 g. nitrogen a day they

calculated that the rate of exchange between the tissue pools was from 15 to 30 g. a day, the quantities varying slightly according to the model assumed for mathematical calculation. Thus, what I have called the "internal circulation" of nitrogen would be about twice as fast as the input and output from the body as a whole.

I would suggest that if the protein stores are depleted, these internal rates of exchange will fall. Therefore the nitrogen available for rapidly metabolizing tissues may be reduced largely irrespective of the dietary intake.

There is possibly some indirect evidence for such a concept. There are situations where a negative nitrogen balance produced by calorie deficiency seems actually to benefit some organs at the expense of others. At the Gambia Conference,[65] Dr. Trowell suggested that the marasmic infant did not have lesions of liver and pancreas because it lived "on its own meat". At the time I could not understand how a creature with an extra drain on its protein stores from calorie deficiency could in any way be better off than one with adequate calories. But now I think that Dr. Trowell may be right: although there may be an increased drain on the body stores as a whole, the breakdown of muscle protein makes more nitrogen available for the rapidly metabolizing organs: in other words, it promotes the internal circulation.

This, of course, is hypothetical, but it might prove a starting point for experimental work.

INFLICTION OF STRESSES

ALLISON: We are very much aware that there may be all kinds of differences between the normal dog and the dog with depleted protein stores. Therefore we have tried to determine what Dr. Elvehjem has called the "optimum adequacy" by putting stresses on these animals. For example, we have given dogs 2-aminofluorene, which is very toxic and is oxidized, we think, in the liver. From the excretion of intermediate products, an estimate was made of the degree of oxidation and detoxication.[66] The dog with full protein stores, as determined by the amount of nitrogen needed to maintain equilibrium, detoxified 2-aminofluorene better than one with lower stores. Then, too, we have used toxic drugs, the ethylene phosphoramides, to measure resistance to stress. These drugs are used to reduce the growth of tumours, but they will inhibit mitosis in, I think, every tissue of the body if given in sufficient concentration. We find that the dog with what we might call high protein stores can be given relatively high dosage for 2 or 3 months without evidence of toxicity, while another animal with lower protein stores will develop a marked leucopenia on the same drug intake.[66, 67] Using that kind of information, we have decided that about 0·2 g. of nitrogen per kg., from whole egg protein or other protein of high nutritive value, is the minimum *adequate* amount of protein for the dog.

MAYER: So you get differences in resistance to toxic agents even within the range of protein intakes in which you definitely have nitrogen balance?

ALLISON: Yes, certainly. You might be interested in another unique type of stress that we are now studying. It is the stress of a growing sarcoma transplanted into the rat. The data in Figure 8 illustrate the growth of the body of the rat in the presence of the tumour. The animals were fed different diets. The weight of the body was calculated by subtracting the weight of the tumour. The sarcoma grew semi-logarithmically, reaching about 1 g. at the midpoint of the curve and weighed approximately 15 g. or more at the end of 20 days.[69] Eventually the sarcoma depleted the body of the animal. The control animals were fed a

diet containing 12 per cent casein. This amount of casein is within the range described yesterday, the diet being deficient in methionine. Adding methionine and cystine improved the growth of the body in the presence of the tumour; interestingly enough, under this stress glycine added to the methionine improved the growth still more.

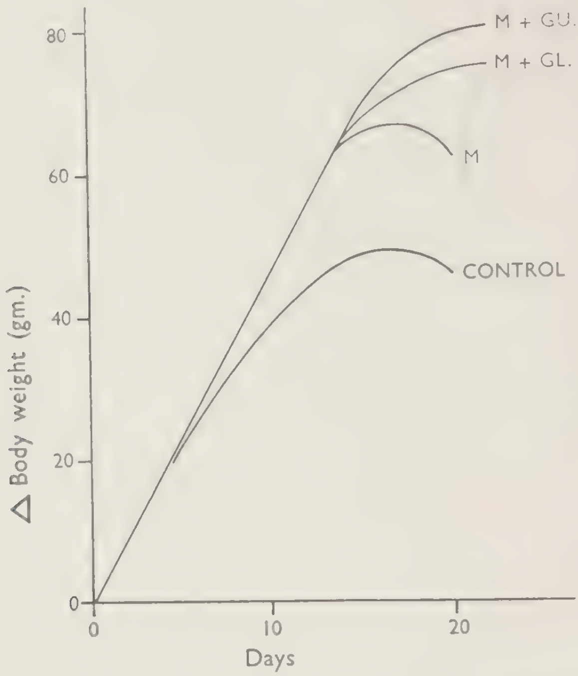

FIG. 8. The increase in body weight of rats fed different diets in the presence of a transplanted growing sarcoma. The control rats were fed 12 per cent casein; others 12 per cent casein supplemented with an optimum amount of methionine (M), methionine plus glycine (GL), and methionine plus guanidoacetic acid (GU).

We have done several experiments of this type which show that under the stress there may be a glycine deficiency, something like that found in the chick. I don't want to over-emphasize the glycine deficiency, but I do want to emphasize that with this stress you can improve the growth of the normal tissues tremendously by proper supplementation with methionine plus glycine. The curve labelled GU shows the effect of guanido-acetic acid. We thought that glycine and arginine might possibly be involved in forming this acid, and indeed guanido-acetic acid improved growth as effectively as glycine.

WATERLOW: Have you any data on the amino-acid concentration in the tumour? Is it raiding the tissues in some special way?

ALLISON: It may be. We hope to measure that, but we have not yet done so.

MAYER: Have you tried any other amino-acids besides glycine?

ALLISON: We have tried alanine and ammonium citrate and ammonium acetate so far, but they have not had as much effect as glycine or guanido-acetic acid. These data were presented to emphasize a type of research involving stress. Diarrhoea, which is another kind of stress, has been mentioned. In some work on hamsters we have found that with certain diets diarrhoea can have a very marked effect on nutrition and even produce kidney damage. I think one of our research approaches for the future should be to determine the requirements of the organism under stress.

MAYER: Dr. Elvehjem's experiments showed that even in growing animals a certain type and amount of protein which permitted growth were not enough to protect the liver against fatty infiltration. Now you present data showing that amounts of protein which are enough to maintain nitrogen balance are not necessarily enough for optimal performance, again presumably of the liver. Do you think that either the appearance or the function of the liver would be a better test of adequacy of protein nutrition than nitrogen balance or even growth rate?

GOPALAN: What liver function test are you thinking of?

MAYER: Detoxication.

PLATT: He has a liver function test in the synthesis of albumin.

ALLISON: We have in mind to use as a test the synthesis of albumin compared with the ability to detoxify.

ENZYME ACTIVITY

ALLISON (continued): It is important to determine how well the animal can meet certain problems biochemically. Figure 9 shows some of the changes that can take place in a protein-depleted liver and illustrates the type of thing that Dr. Elvehjem is also doing. These data were obtained in the rat[61]; unfortunately

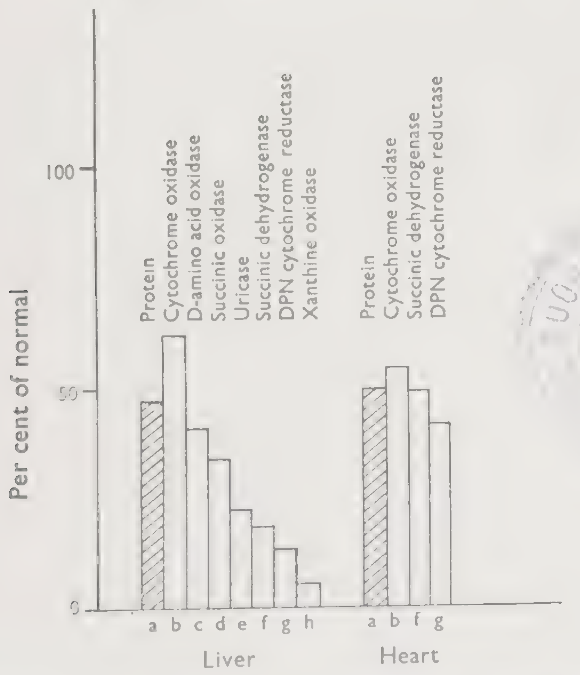

FIG. 9. The bars with slanted lines (a) illustrate the loss in total protein, expressed as per cent of normal, in the liver and heart of protein-depleted rats. The effect of depletion upon certain enzyme systems is also illustrated.

we do not have observations of this kind on the dog. The liver protein was reduced by about 50 per cent, and the figure shows the relative activity of different enzymes in the depleted liver, as compared with the normal.[70]

Co-enzyme A was also reduced in these animals. In the dog we found that we could detect this reduction in co-enzyme A by the ability of the liver to acetylate 2-aminofluorene.[58] Maybe we could use the acetylation of sulphanilamide in man to measure the activity of co-enzyme A. I think a very good possibility for

future research is to develop methods for determining the activity of enzyme systems in the liver *in vivo*.

ROSE: These animals were depleted?

ALLISON: Yes, they were very badly depleted. With the xanthine oxidase as low as that, you would consider them very badly depleted animals, would you not, Dr. Elvehjem?

ELVEHJEM: Yes.

TERROINE: If we want to use enzyme activity in the liver as one of our indices of good protein nutrition, we must be very careful what enzymes we choose to take as a basis for our calculation. It is clear from the figure given here, and indeed from nearly all the studies published in this field, that enzymes can be divided into two large categories: one group, of which cytochrome oxidase is an example, that is very insensitive to nutritional conditions; and another group which, on the contrary, is extremely sensitive to all nutritional stresses—for example, xanthine oxidase. I might say that Mlle. Terroine is at the moment engaged in a systematic investigation of this problem. If we want to use enzymes as an index of nutritional adequacy, we must, I think, choose the ones that are most sensitive and not the others.

WATERLOW: In man, as far as our observations go, DPN-cytochrome-c-reductase, which, functionally, is an extremely important enzyme, falls into the same category as cytochrome oxidase, and is not reduced by malnutrition.[71]

ELVEHJEM: I would like to throw out another caution. These are all new studies on changes in enzyme systems, and we do not know what they mean. I would be opposed to trying to use them clinically or as an assay of nutritional adequacy, because I think we need to learn much more about them. That seems to be the trend in all research, that if something new comes out, everyone over-emphasizes its value. I agree with Professor Terroine that we must be very careful in the interpretation of enzyme changes in tissues. Nevertheless, I agree that we need to go ahead and study these changes, and, perhaps, after a certain length of time, we may find a pattern which means something. There is no question but that we get these changes in animals, but as Dr. Waterlow has shown, in man you may get quite different results. You can't find xanthine oxidase at all, can you?

WATERLOW: Xanthine oxidase, amino-acid oxidase and choline oxidase—all enzymes which have been interesting in animals—are so low in the human liver that I have been unable to measure them.

ELVEHJEM: So we must be very careful in our interpretations. But that does not mean that we should not go ahead with the studies.

WATERLOW: Would you agree that when you find a reduction in the activity of an enzyme, it must be taken as something significant, something indicating an abnormality, which ought to be investigated?

ELVEHJEM: Well, I don't know whether or not a low xanthine oxidase has any ill-effect on optimum nutrition. Maybe it is advantageous. We don't know what the optimum level is.

DARBY: Are we certain that the finding of a low xanthine oxidase or any low enzyme activity is the result of lack of protein, or can it be due to the lack of something essential for the formation of prosthetic groups?

ELVEHJEM: It may be that, or it may be lack of another enzyme needed for the formation of the enzyme protein.

WATERLOW: Or it might be a mineral.

PLATT: Yet the rat lives for the first ten days of its life without any measurable amount of xanthine oxidase at all.

TERROINE: I agree that a reduction in enzymes need not necessarily entail physiological consequences. It has been discovered in studies on rats carried out recently in the United States that the lack of xanthine oxidase in the liver did not reduce the production of allantoin, but on the contrary increased it. It is possible, therefore, that some enzymes may take the place of others when one of them disappears, and this is especially true in the case of purine metabolism.

We are faced here with two problems. One is to ascertain whether a reduction in the amount, or the total disappearance, of a certain enzyme can have harmful effects; the other problem is to use the variations in the concentration of enzymes as an indication of nutritional status, without drawing any conclusions about the physiological results of these variations. In this connection, I think that the warnings of Dr. Elvehjem, and other studies now being carried out in France on the same subject, are of extreme importance for us all.

ALLISON: We must also emphasize the great importance, for the formation of liver enzymes, of having enough pantothenic acid and riboflavin in the diet, as well as protein.

NUTRITIVE VALUE OF DIFFERENT PROTEINS

ALLISON (continued): Another point that we have been interested in is the optimum relationship between caloric intake and nitrogen intake with various dietary proteins. We did experiments in which protein intake was constant but the caloric intake was varied, giving the data illustrated in Figure 10.[72] Under these experimental conditions there was a gradual increase in the excretion of urea nitrogen and a drop in nitrogen balance as the caloric intake was reduced.

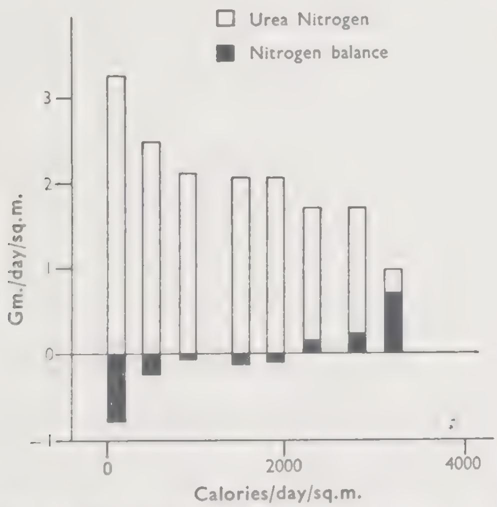

FIG. 10. The effect of varying the caloric intake on nitrogen balance and urea nitrogen excretion in dogs fed a constant casein intake (3·82 g. nitrogen day sq.m.). (See Rosenthal and Allison.[72])

The data in Table 17[69, 73] illustrate the effect on growth and nitrogen retention of feeding different types of protein to beagle puppies. The same amount of nitrogen was added to each diet, and they were allowed to eat these diets *ad lib*. The gain in body weight per g. of nitrogen intake was about the same in

all three groups of puppies, but the ratio of nitrogen balance to nitrogen intake was quite different, reflecting, we believe, the nutritive value of the protein.

TABLE 17

Average increase in body weight (B.W.) per gram of nitrogen intake (I.) and average nitrogen balance (B.) per gram of nitrogen intake (I.) in beagle puppies during fast-growing period

Protein source	B.W./I.	B./I.
Whole egg ..	9·0	0·50
Casein	9·8	0·43
Wheat gluten ..	8·8	0·18

(Table taken from the original, Table 2, Allison, *Fed. Proc.*, **10**, 680, by kind permission of the publishers.)

GOPALAN: The conventional method of measuring the relative value of proteins is, of course, by their effect on growth—that is, on weight gain. These figures show very clearly that we must know to what that weight gain is due. Is it caused by an increase in fat, in water or in cell solids? For example, in certain experiments that we have recently been doing, we found that if we went just by growth, that is, by overall weight increase, the difference between two proteins may appear to be very significant, but when we did studies of body composition and took only the increment in cell solids into consideration, and not the fat, water and minerals, much of the difference disappeared. I would therefore like to know how far this growth method is really a criterion of the nutritive value of proteins.

ELVEHJEM: When you are down in this range, it doesn't make any difference whether you use growth, nitrogen balance or the enzyme content of the liver or tissues; I think you are going to get about the same results. When you get close to the borderline, where the differences are very small differences, I think you can show them up by some techniques more easily than by others. But which is the best for the human, I don't know.

However, I think it is important that when the intake of protein is below normal and growth is less than maximal, some fat begins to accumulate in the liver. This may be due to a lack of threonine, whereas the reduced growth may be due to a minimum intake of some other amino-acid. In each case, each amino-acid may have a different effect on different tissues. In other words, there is perhaps no single criterion.

CATRON: I agree that growth is not always the best criterion. We have an example of this from a study of the calcium and phosphorus requirements of swine. In the baby pig, bone opacity, blood phosphatase and femur ash are all excellent criteria of phosphorus availability, but growth is a relatively poor criterion.

ALLISON: I would like to emphasize that it is perfectly possible, and people do it all the time in designing experiments in the laboratory, particularly with rats, to obtain good correlation between protein efficiency and nitrogen retention. But your point is very important; weight gain, unlike nitrogen retention, certainly can be misleading, because of changes in body water, fat or other variables. I presented these data to illustrate a situation where the body weight gain was very definitely *not* correlated with nitrogen retention.

MAYER: Drs. Eppright and Jebe (Iowa State College) have data on growing children[74] which are very similar to those in your table. They found that children on a low protein diet—if you can have a low protein diet in Iowa—have a tendency to be very much fatter than children on the high protein diets.

WATERLOW: What were the calorie intakes on your three diets, Dr. Allison?

ALLISON: They were about the same: if anything they were a little higher on the wheat gluten diet, which surprisingly enough the puppies tended to eat better than the whole egg diet.

WATERLOW: If the intakes were equal, the animals on the egg protein diet must have been using up more calories, while those on wheat gluten stored some as fat, and expended less.

MAYER: Was there a difference in spontaneous activity on the different diets?

ALLISON: Very definitely. The animals fed the egg diet were more active than those fed the wheat gluten diet; they were also leaner and more lanky.

Next, I should like to present some comparative observations on the amounts of different proteins needed for growth and for maintenance. The data in Table 18[68, 75, 76, 77] were determined by a group of co-operating laboratories using the same samples of proteins. I put the results in terms of egg white as 100, because egg white stood at the top in nutritive value in these studies. Whole egg was not at the top, primarily, I believe, because of the effect of processing upon the sulphur amino-acids. Two laboratories reported on growth in the rat, while three laboratories reported the relative amounts necessary to maintain nitrogen equilibrium in the rat, dog and man.

TABLE 18

Nutritive value of six selected proteins, expressed relative to egg-white as 100.[68, 75, 76, 77]

Animal	Egg-white	Whole egg	Beef	Casein	Peanut flour	Wheat gluten
			For growth			
Rat	100	95	84	80	31	8
Rat	100	90	78	71	56	41
			For maintenance			
Rat	100	87	73	54	49	69
Dog	100	76	68	64	49	39
Man	100	103	74	74	62	46

TERROINE: These figures bear out the point made earlier, that differences between the biological values of various proteins are less accentuated in the case of man than they are for small animals. Hence, if we base our calculations on small animals, especially those with a rapid rate of growth, we can be quite certain that the coefficient of correction which we adopt will be an excellent one for man.

ALLISON: It is interesting that wheat gluten, which is quite deficient in lysine, apparently is as good as casein for maintenance in the adult rat but not for growth in the young rat. The data on the adult rat were obtained by Dr. Mitchell and Dr. Beadles[77] who have demonstrated that lysine is not essential for maintenance in this animal. I would like to emphasize, however, that if there is any depletion at all, so that you have to replete tissue proteins, then

the nutritive value of wheat gluten is low, as in the growing rat. I believe that repleting the tissue proteins in the depleted adult presents problems similar to those of growth. This concept might have some application in countries where adults as well as children may be chronically malnourished, with depleted protein stores.

KING: I think it is a most important idea that has come out of this discussion: that an organism depleted of protein may be able to maintain itself *in statu quo*, at a certain equilibrium level. Yet, compared with the normal, it shows changes in biochemical behaviour—in the activity of enzymes, in the response to stresses of various kinds, and in the pattern of amino-acid requirements. Of course, all those who have considered the problem of protein requirements have had in mind the possible effects of stress, and have therefore tended to allow a safety margin in their figures. But, as we all admit, much of that is guess-work. Dr. Allison has shown what a large gap there is between the maintenance level in the depleted dog and in the well-fed dog. We might take these as corresponding to minimum and optimum levels. This type of experimental approach will perhaps enable us eventually to define these levels with equal precision in man.

CHRONIC PROTEIN MALNUTRITION

PLATT: I believe that, in practice, we are generally dealing in man with a form of protein malnutrition which may have persisted through several successive generations. It is useful therefore to produce the same condition in animals in order to compare the results of chronic and acute deprivation. We have been studying rats which have not only been deprived of protein themselves, but born of mothers which also, for at least the early part of their lives, have been deprived of protein.

We find that on a basal Gambia-type diet* the mothers usually eat their offspring and the ones that do survive do not thrive.[79] If, however, from the time of mating (when the females are 21 weeks old), the potato starch in the basal diet is replaced by 2, 5 or 10 per cent of fishmeal, then the reproductive performance improves and in animals on the 10 per cent fishmeal supplement it approaches that of the animals on the stock diet (diet 41 supplemented with liver).

Although the introduction of fishmeal into the Gambia-type diet has enabled us to produce a third generation (i.e. not including the deprived mothers in generation 0), there were, even when the basal diet was supplemented with 10 per cent fishmeal, heavy losses of young rats at weaning at 3 weeks. In one experiment, in the first week after weaning, 14 out of 29 offspring died; in the following week, 3 out of the remaining 15 died. In the following generation, instead of weaning at 3 weeks, the young rats were allowed to run with their

* Composition of 'Gambia-type diet':

Wholemeal flour	80 parts
Potato starch	10 ,,
Haricot bean meal	5 ,,
Lucerne meal	2 ,,
Groundnut oil	2 ,,
Salt mixture (Loureiro)	1 ,,

Vitamin A 700 I.U. per 100 g. diet.
Vitamin D 350 I. U. per 100 g. diet.
Vitamin B complex mixture[78] 0·03 g. per 100 g.

mothers and have access to the mothers' food for 2 weeks after the normal time of weaning. For the first week the mother was given the 10 per cent fishmeal diet, for the second week the basal diet. In 22 young there were no deaths.

Table 19 summarizes some observations on reproductive performance.

TABLE 19

Effect on reproductive performance of supplementing basal 'Gambia-type' diet with fishmeal

Diet	Average wt. at weaning (g.)	Average wt. of dam after parturition (g.)	Time before birth of first litter (weeks)
Basal	usually dead	118	26
Basal + 2 per cent fishmeal ..	14·6		
Basal + 5 per cent fishmeal ..			16
Basal + 10 per cent fishmeal ..		149	5½
Stock	34	218	4

The maternal diet affects not only the weight of the baby rats at birth and at weaning; the weight of the young on the basal diet was significantly less at 15 weeks of age than that of animals on the 10 per cent fishmeal diet. Young animals born of mothers who had been on a low protein diet were only about a third of the weight of stock animals of the same strain at the same age. Growth of young animals, measured by weight gain, is slower with each successive generation.

WATERLOW: Have you done any experiments on other animals besides rats?

PLATT: Mrs. J. Miller in my Unit has worked with piglets, and produced in them some of the pathological changes seen in man that are not usually found in the rat.

These results in rats illustrate some of the qualities of a diet based on a plant food economy, judged by its effects on growth and reproduction, the two physiological processes in which the supply of good protein is specially needed; the advantages of a mixed animal protein supplement are clear-cut. The experiments also show that, for the infant of the protein-malnourished mother, continued feeding with maternal milk during the period of "mixed feeding"[80] is a life-saving measure.

In case I may be misunderstood, let me say here that I do not decry the value to the young animal of the foods other than breast milk that may be given; it has been shown[81] that the suckling rat grows better when it eats some of its mother's food. I do strongly urge, however, that the nutrition of the mother in the under-developed community be given the fullest consideration, both for her own sake and for the sake of the health of her children.

RELATION OF PROTEIN TO CALORIE INTAKE

WATERLOW: There is one other point that I should like to raise. We know that if the supply of calories is inadequate, the animal or human being will tend to go into negative nitrogen balance. Dr. Allison showed some data on

this. In all our discussion on protein requirements we are assuming that the need for calories is adequately met. But, as Munro has shown in a recent review,[82] the nature of the relationship between calorie intake and nitrogen metabolism is by no means clear. Carbohydrate spares protein, but by what mechanism? We don't know. Moreover, Munro quotes some evidence to show that the source of the calories may be important: that fat may be more effective than carbohydrate. Therefore—though it is an obvious enough point—we can't consider protein needs without taking into account the rest of the diet.

ELVEHJEM: We have some experiments that bear on this. Consider Dr. Aykroyd's hypothetical child 2 years old, weighing 10 kg., and getting 1,000 calories and 3 g. of protein per kg. If we calculate the protein intake in mg. per calorie, we get a figure of 30. Now we did some experiments in which rats were fed at three different levels of protein intake. At each level, the ratio of protein to calories was kept constant, at 12, 24 and 49 mg. per calorie. Dr. Aykroyd's child therefore falls within the range of these experiments. At each protein level, four different diets were fed, containing different proportions of carbohydrate and fat. Of course, as the percentage of fat increased, the proportion of protein also had to be increased in order to keep the ratio of protein to calories constant. The results are shown in Table 20.

TABLE 20

Growth and deposition of liver fat in weanling rats fed diets varying in fat and carbohydrate at three protein/calorie levels

Group no.	Diets			Protein (mg. per calorie)	Wt. gain (g. per 3 weeks)	Liver fat (per cent wet wt.)
	Casein (per cent)	Fat (per cent)	Sucrose (per cent)			
1	4·8	1·0	90·4	12	12	13·6
2	5·0	5·0	86·0	12	12	10·7
3	6·7	33·3	54·7	12	17	13·2
4	9·6	82·7	0	12	35	15·8
5	9·5	1·0	85·7	24	72	8·5
6	10·0	5·0	81·0	24	74	6·8
7	13·3	33·3	48·1	24	87	5·2
8	18·2	74·5	0	24	105	8·1
9	19·0	1·0	76·2	49	112	3·0
10	20·0	5·0	71·0	49	133	3·2
11	26·7	33·3	34·7	49	121	4·1
12	33·0	60·4	0	49	117	5·7

On 12 mg. of protein per calorie (groups 1–4), with high levels of carbohydrate, growth was very poor and the livers were fatty. When the proportion of fat was increased, growth and protein efficiency improved, but the liver fat was still high. When the ratio of protein to calories is doubled (groups 5–8), increasing the proportion of fat again causes an increase in growth and in protein efficiency. The liver fat is lower, but still not normal. I think this shows clearly that a high proportion of fat has a significant sparing effect on protein.

But when you again double the ratio of protein to calories (groups 9–12), you get no sparing effect from fat; there is plenty of protein, and so any sparing effect of fat does not produce any further improvement. At this level of protein, liver fat is now normal, and this might be regarded as the optimum level of protein intake. These results also suggest that for certain amino-acids the demands of growth may take priority over the need for keeping down the liver fat.

GYORGY: The caloric intake was not identical in the different groups?

ELVEHJEM: No.

GYORGY: Can you be certain of excluding a higher caloric intake as the cause of better growth and better nitrogen retention, if the diets were not iso-caloric? I don't see how you can.

ELVEHJEM: The total caloric intake must go up if the animal is going to grow.

GYORGY: I am not so sure whether the fat as such did it, or the high caloric intake.

ELVEHJEM: Well, the caloric intake is the same per mg. of protein. Moreover, I doubt if the high fat diet is any more appetizing than the sucrose diet, so I don't think it is merely a stimulation of appetite. How closely you could extend these results from rats to human infants, I don't know, but at least we are in a right range of protein intake per calorie. They emphasize the sparing effect of fat when protein is low, and the absence of a sparing effect when the protein intake is fairly high.

KING: Perhaps, Dr. Catron, you would now take up the discussion, and give us your experience from the field of animal nutrition.

Animal Husbandry

PROTEIN REQUIREMENTS OF PIGS

CATRON: Most of what I have to say will be on the nutrition of swine, because that is my own research field. At Iowa State College we have been studying swine nutrition on a life-cycle basis for 10 years. In the last 5 years we have been interested primarily in re-evaluating the protein requirements of swine, in the effect of antibiotics, and more recently in the nutritional requirements of the baby pig.

We can now wean the baby pig from its mother at 1 week of age, or at a minimum weight of 5 lb., and put it on a dry ration, and bring it up to 50 lb. at 8 weeks, with less than 5 per cent mortality. We do this on 80 lb. of feed; or 1·6 lb. of feed per lb. of gain.

We have established a 1–5 week growth-index of weight gain under very well controlled, air-conditioned baby pig laboratory facilities. Taking the growth-index on soya protein and lactose as 100, on other diets we get the following results:

soya protein and starch:	about 70
casein and starch:	,, 120
casein and lactose:	,, 158
dried skimmed milk:	,, 169

In other words, there is a small difference between dried skimmed milk and our recomposed milk of lactose and casein, but without the lactalbumin. I should add that we put in about 10 per cent sucrose in these diets, and also fats, vitamins and minerals, based on the baby pig's requirements as near as we know them.

GYORGY: What happens if you use any other sugar—say only glucose or sucrose instead of lactose?

CATRON: Our carbohydrate of choice for the baby pig is lactose; then a 42-dextrose-equivalent corn-product, then sucrose, ground yellow-corn, dextrose, raw corn-starch—and so on down.

GYORGY: Your first choice is lactose because it is the best for growth?

CATRON: Yes.

GYORGY: On the same food intake?

CATRON: We don't know. They are fed *ad lib.* We are going to test it on an equal intake. We want to know what lactose does for the pig. As far as energy is concerned, there isn't any reason why you can't get it from one carbohydrate as well as another. So the lactose must be having some effect on the microflora in the intestinal tract, besides supplying energy, and so we are attacking it from a bacteriological as well as a nutritional aspect.

GYORGY: Do the pigs get diarrhoea?

CATRON: No, sir: a soft stool, but not diarrhoea. We ourselves were very surprised to find this.

GYORGY: Well, the human infant on the breast has only lactose as the source of carbohydrate.

CATRON: Now here is an interesting thing in these baby pigs. Between 4 and 5 weeks of age there is a turning-point. Before that time, from 1 week up to 4 weeks, soya protein, regardless of how we have had it prepared in terms of time, temperature and moisture, has given us very poor performance. But after 4 to 5 weeks it is an excellent source of protein for the pig.

TERROINE: Do you cook the soya?

CATRON: Yes, it was heated.

DEAN: Was the trypsin inhibitor destroyed?

CATRON: The industry knows a good deal about the processing of soya protein for animals, and they destroy the trypsin inhibitor and the urease activity. It is a good quality protein when compared with fishmeal, both for pigs and poultry.

SCRIMSHAW: You say there is a turning-point at 5 weeks. What is 5 weeks equivalent to in the child?

PLATT: You wean at 8 weeks with a pig, don't you?

CATRON: That was the old rule of thumb that has been arbitrarily set by man.

PLATT: Well, man, before any arbitrary rule was set, weaned at about 2½ to 3 years. If you take half of that, you are about right.* That would put it at about 18 months.

CATRON: We think that up to 4 or 5 weeks the pig has an incomplete enzyme system for handling soya protein. We can't believe that the ration is short of amino-acids, otherwise why does it do so well after that? We are very much interested in this, and we want to relate it to infant nutrition. These results suggest that soya protein may not work as well in food for young infants as it does later.

DEAN: Your data, then, would support Dr. Gyorgy's old-fashioned idea, that the best food for a small human child is human milk, and the best food for the sow's child is the sow's milk?

* From the weight-growth equivalence data given by Brody,[83] I reckon that a day in the life of a piglet is equal to ten in the life of a child. However, as Brody points out, if the ages of man and other species are made equivalent after puberty, they do not coincide before puberty. (B. S. Platt.)

CATRON: There isn't any doubt about that. Sows have been milked and the milk fed back to baby pigs. We have never been able to synthesize a diet comparable to sow's milk. The only limitation in sow's milk is quantity—that is, if one is thinking in terms of amino-acids and proteins.

PLATT: Would you expect that in time you will be able to do without milk—I don't mean just sow's milk but milk of any kind—after the first 3 or 4 days, up to the age of 4 weeks when your troubles seem to get less?

CATRON: At the moment my answer is, I think so.

PLATT: But you are not prepared to speculate now on what might replace milk?

CATRON: No. So far we have been unable to get the same performance out of plant protein diets in the first 4 or 5 weeks.

PLATT: But need it be plant protein?

CATRON: No, it need not be. It could be fishmeal or animal protein. But, by and large, the animal protein available for pig-feeding in this country is not of best quality.

AYKROYD: What about fishmeal?

CATRON: We haven't done any work with fishmeal at low levels with the young baby pig.

PLATT: Do you breed from the animals which you start on an artificial regimen from 4 days after birth?

CATRON: Yes.

PLATT: And does the substitution of artificial feeding for mother's milk make no difference?

CATRON: We are improving reproduction performance. In their second and subsequent litters, gilts are producing an average of 11·4 pigs per litter; the best we ever got before was 8·2.

PLATT: The age at which mixed feeding is introduced is of some importance. It probably is not well-known that even the laboratory rat is taking some of its mother's food as well as her milk for some time before it is weaned and that it does not put on weight so well if it is denied access to its mother's food. Murphy and Dunn[81] conclude from their study of the growth of young rats that "The shape of the preweaning growth curve of the rat is profoundly affected by the lack or availability of solid food".

WATERLOW: How does the artificially fed pig grow after 5 weeks?

CATRON: Whereas we can produce a 50 lb. pig at 8 weeks, the sow really does well to produce a 35–40 lb. pig at the same age.

GYORGY: What is the composition of your pig when it reaches 50 lb., compared with the sow-fed pig?

CATRON: Quite similar, in terms of protein.

MAYER: The trouble is that you are breeding an animal to eat it, whereas what we are interested in is prolonged physiological performance.

CATRON: Well, I agree. Our main objective is rather different from yours. We want maximum weight gain at minimum cost. Our objective now is to produce as many pigs as we can per sow, and a pig that will grow fast and yet produce a lean carcass. But it seems to me that in the early stages our aims are not too different: in the discussion on infant requirements you also were using weight gain as your criterion.

Now here is a problem that I think has deep implications in the human field. Many workers have shown that as we increase the protein of the ration we increase the leanness of the carcass, whether expressed as per cent of lean cuts

or in terms of specific gravity. European swine-feeding, especially in Denmark and in England, calls for much more protein than we feed here in the United States. How much protein can we afford to feed to get a leaner carcass? That is our big problem.

GYORGY: What is the efficiency of the protein in the food, expressed as protein laid down in the pig?

CATRON: Depending on whose data you take, it is between 14 and 22 per cent. One of the things that has impressed us, as we strive towards a balanced diet, is that with trace minerals and vitamins and antibiotics we can just about cut in half our protein needs, in terms of live-weight gain and feed efficiency. This does not take into consideration factors like disease resistance and so on.

SCRIMSHAW: It does have important implications for man.

CATRON: Yes, in that if we get a balanced diet, we ought to be able to get along with a little less protein.

MAYER: What trace minerals do you give?

CATRON: We give iron, copper, cobalt, manganese, zinc and iodine.

PLATT: Why do you have to add those? Are they not present at all in the natural diets?

CATRON: Yes, but it depends not only on the quantity present but also on the availability of some of these nutrients. Zinc, for example, has been one of the trace elements that we used to think was adequately supplied by natural diets, but more recent research indicates that when deficient it may be involved in a disease which we call parakeratosis in pigs.

PLATT: Are your proteins concentrated from foods or are they whole, natural foods?

CATRON: All this work is based on a corn-soya diet.

PLATT: But you don't give greenstuffs, which might supply some of the minerals and vitamins?

CATRON: No, we don't add any alfalfa meal or legume meal.

EFFECT OF ANTIBIOTICS

CATRON (continued): We are also considering other factors, such as the influence of antibiotics, drugs and hormones. With antibiotics added we get optimum growth and feed efficiency on a ration containing 14 per cent protein, but without the antibiotics we need 16 per cent to get the same performance.

SCRIMSHAW: Do you get less effect from antibiotics as your sanitation improves? I ask that because, in children in Central America, we apparently do get definite growth-promoting effects from aureomycin fed orally (50 mg. a day) in some circumstances, but not in others.

CATRON: We have not had any response from the feeding of aureomycin to the growing-finishing pig—from 30 lb. to market weight—since 1952.

CRUICKSHANK: Does that coincide with improved sanitation?

CATRON: Probably. I think that the work on poultry and on swine would confirm that. It has been shown at the germ-free laboratory at Notre Dame, that on a complete diet you don't get much effect by feeding antibiotics.

GOPALAN: Do you observe any variation depending on the type of protein you employ?

CATRON: Work all over the country indicates that there is a difference. The antibiotics produce a greater improvement of growth with peanut protein than with soya protein.

TERROINE: On the whole, French techniques of rearing pigs are pursuing exactly the same aims as you have outlined. We also do not want to have very fat pigs. We want them to weigh only between 50 and 80 kg., and to be as lean as possible. We too try to feed the pigs without the sow, and aim at the largest litter possible without attention to the quality of the milk; in fact, we too rely on artificial feeding.

Another point with which we are in full agreement is that if the feed is well balanced, and of a suitable nature for the animal in question, antibiotics have only a very slight effect, or none at all.

CATRON: That, we think, depends somewhat on the disease level. The higher the disease level, the more benefit you get from feeding antibiotics. We have shown unequivocally by balance studies that you can spare vitamin B_{12}, niacin, pantothenic acid, and riboflavin by feeding 5 mg. of a broad-spectrum antibiotic per lb. of total diet. In other words, we can get along with less of those nutrients in the diet.

FREMONT-SMITH: Do you know why?

CATRON: No.

DARBY: You can get this sparing effect although you get no difference in growth?

ELVEHJEM: You do get a significant difference in growth if you have a limiting amount of one or more vitamins.

DARBY: But let's assume that Dr. Catron's pigs are clean pigs. Despite the fact that you have no infection, do you still get this sparing effect?

CATRON: Oh, yes. That is why I maintain that the effect of antibiotics is both a disease-control effect and a nutritional effect.

ELVEHJEM: You asked why, Dr. Fremont-Smith. If you alter the intestinal flora, you may destroy those organisms that utilize the vitamins, and may favour those organisms that synthesize the vitamin.

FREMONT-SMITH: Have you been able to eliminate the possibility that it acts elsewhere in the body?

ELVEHJEM: The work at Notre Dame shows that you don't get these effects in the sterile animal.

FREMONT-SMITH: You don't get growth, but won't it spare vitamins?

ELVEHJEM: No, it will not.

FREMONT-SMITH: But I think there is evidence of an internal effect of the antibiotic within the organism. Dr. Diaz and Dr. Ephraim Shorr at Cornell Medical College have shown that aureomycin, in addition to its antibiotic action, has a specific effect on the enzyme systems in the liver which regulate ferritin metabolism. They have been able to show that aureomycin specifically protects these enzyme systems against certain forms of stress in the living animals, and *in vitro* it protects liver slices against the stress of anaerobiosis. This applies whether the aureomycin is given by mouth or through the portal vein.

ELVEHJEM: I would like to question that, because if it is introduced in the blood stream, it undoubtedly gets into the intestinal tract.

FREMONT-SMITH: The *in vitro* experiments can have no bearing on the bacteria in the gut.

TERROINE: I should like to mention some work being done in France by M. Calet, in the Laboratory of Nutritional Biochemistry at the National Institute for Scientific Research. He has investigated the mechanism of the action of antibiotics by balance studies. The balances indicate that the increase in weight shown by the animals cannot be explained by a higher degree of

nitrogen retention, but rather, in many cases, by a greater storage of fat in the body. Is this caused by a reduction in energy expenditure, or by an increase in fat production? We do not know. But these studies on rats show that one of the effects of aureomycin is to stimulate in some way or other the storage of fat.

CATRON: We have been very much concerned over that. Earlier workers in this country reported that the feeding of antibiotics made pigs fatter, but we have never been convinced that this was true. All our work on protein levels has been done with and without an antibiotic to check the effect on the leanness or fatness of the carcass. We make an incision on the back of the pig and insert a little ruler and measure the thickness of fat. The pig doesn't seem to mind that as much as you would think. Recently workers at Purdue University and Iowa State College have developed an electrical technique for measuring this. They can hold this little machine above the pig and read on a needle the amount of backfat. I think that might apply in the human field also.

The only difference, with and without the antibiotic, that is significant statistically is the size of the loin muscle. At the lower levels of protein—and this may explain the results in rats mentioned by Professor Terroine—antibiotic feeding seems to give a fatter carcass and lower specific gravity, but, as we increase the protein, the effect becomes less.

PLATT: The antibiotics may act, I suppose, by sparing protein from loss by microflora.

CATRON: Yes, it is possible, since the bacteria in the intestinal tract require nitrogen. But when you start feeding an antibiotic, you get a transitory decrease and then a tremendous, manifold *increase* in total microflora count.

PROTEIN REQUIREMENTS OF DIFFERENT ANIMALS

CATRON (continued): Now, to finish, I would like to indicate what we think are the crude protein requirements of different animals at different stages of life. These are shown in Table 21. All these figures are for maximal performance; there is no question of minimal adequacy. We have worked out a slide rule called a Pork Costulator, by which we can take the price of corn and the price of supplement—the supplement being protein with minerals, vitamins and antibiotics added—and tell you the least-time ration—the one that gives you the fastest gains—or we can tell you the least-cost ration, the one that gives you the cheapest gains.

ELVEHJEM: If human requirements at different ages are proportionately the same as those you give for the pig in the table, and if the human adult requirement is 1 g. per kg., then the requirement for lactation would be 1·6 g. per kg., and for the period of fastest growth it would be 2·4–2·8 g. per kg., which is very close to the figure put forward by the paediatricians.

CATRON: Table 21 also shows the requirements of ruminants. As you all know, ruminants can obtain one third of their protein needs from non-protein nitrogen or urea. Therefore if we humans want more animal protein, the ruminant offers a great opportunity for the upgrading of poor quality feeds and roughages to protein of higher biological value. The trouble is that in the world as a whole there is a deficit of crude protein for animal feeding. We have made a search of the literature, and on the basis of the best figures we could find, we estimate that for the whole world there is an annual deficit of 7 million tons of crude protein. This is based on the actual or estimated livestock population at the present time, and does not take into account the rapid increases in some areas.

TABLE 21

I. Protein requirements of non-ruminants

Class	Stage of life cycle	Crude protein required (per cent of total ration)
Swine	Pre-gestation and gestation	14
	Lactation	16
	Pre-starter (up to 12 lb.)	24
	Starter (12–25 lb.)	18
	Grower (25–50 lb.)	16
	Growing-finishing	
	50–100 lb.	14
	100–150 lb.	12
	150 lb. to market	10
Poultry		
Chickens	Starter	20
	Grower	16
	Egg production	15
Turkeys	Starter	28
	Grower	20
	Finisher	16
	Egg production	16
Horses	Colts (to 2 yr.)	12
	Lactating mares	12
	Mature horses (work)	9

II. Protein requirements of ruminants

Dairy cattle	Calves	15
	Heifers	12
	Dry cows	12
	Lactating cows	14
Beef cattle	Calves (to 500 lb.)	12
	Steers (500–1,000 lb.)	11*
	Breeding cows	9*
Sheep* †	Ewes (gestation)	10
	(lactation)	12
	Feeder lambs	
	(1st 30 days)	11
	(after 30 days)	9–10

* $\frac{1}{3}$ total protein can be NPN (urea, etc.).
† $\frac{1}{2}$ protein consumed deposited in wool.

KING: This brings us on to the problem that will occupy us in the later stages of this conference. In this first session we have considered the help—and the warnings—to be obtained from experimental work in animals and man. In the next session we shall discuss human requirements as they arise in life, and not under experimental conditions. In the last session we must try to develop ideas about the fulfilment of these requirements in practice.

Summary

The problem of defining the protein requirement of man or any other species hinges on two things: first, the need to take account of quality as well as quantity; secondly, the criteria to be used for judging whether a given intake is adequate or not.

The question of quality was considered first. Since the reason why proteins differ in their biological value presumably lies in their amino-acid composition, the whole problem would be simplified if it were possible to determine the requirements for each of the individual amino-acids. This is the task that has occupied *Rose* for many years. He has used as subjects normal adult males. The plan of his experiments was to feed a complete amino-acid mixture, and to reduce one of the components by successive steps until the subject went into negative nitrogen balance. The least amount needed to maintain positive balance was considered to be the "minimal requirement". Each subject gave very reproducible results in successive tests, but there was much variation from one subject to another. For this reason, the "safe" level, which should be adequate for all or almost all subjects, was taken to be twice the minimal level. In a long series of experiments the requirement was determined for each of the essential amino-acids in turn, and also for total nitrogen. The least amount of total nitrogen needed to maintain positive balance was 3·5 g. per day—a surprisingly low figure.

When it comes to applying these results to the practical problems of nutrition in the field, a number of difficulties arise. First, nitrogen balance could only be secured if a very large calorie intake was fed. No explanation has been found for this phenomenon, but it immediately suggests that the experimental conditions were in some way artificial. Secondly, the test periods lasted only a week, and therefore no conclusions could be drawn about whether the minimal or safe intakes would be adequate over longer periods. Thirdly, the results were obtained on young men in good health, and cannot be applied to subjects of different age or sex. Lastly, as many members of the group pointed out, the criterion of nitrogen balance is not necessarily sound. These points reflect a real practical difficulty; Rose does not deny the limitations of his work, yet it would be an almost impossible task to extend it to cover all the variables met with in everyday life. Apart from its theoretical importance, the main practical value of these experiments is perhaps to provide one fixed point in the scale of requirements: this fixed point might be taken as the lower limit of the scale, since the physiological and environmental conditions were as far as possible optimal.

Holt, in his work on the amino-acid requirements of infants, had this advantage, that he was able to use growth as a criterion of adequacy. The babies were fed amino-acid mixtures, in which the amount of one was varied, the others being kept constant. The minimal adequate level was taken to be the least amount which supported satisfactory growth and nitrogen retention. No strict definition of "satisfactory" is given, and again, since each test period only lasted a few days, it is uncertain how far the results could be extended over longer periods. A puzzling feature of these experiments is that when the intake of one amino-acid is reduced to zero, and growth stops, there may still be a positive nitrogen balance.

The amino-acid requirements of infants, determined on this synthetic diet, were some ten times higher per kg. body weight than Rose's figures obtained by essentially the same method in adults. However, the results corresponded fairly closely with the actual intakes of breast-fed babies who were growing well. *Gyorgy* made the point that we must not think of proteins only in terms of amino-acids. Intermediate degradation products, such as peptides, may be of importance for man as they are for some micro-organisms.

The ability to cure kwashiorkor is an even more severe test of a diet's effectiveness than its ability to promote growth. *Hansen* obtained successful results in the treatment of kwashiorkor with a mixture of amino-acids. However, because of the number of variables that affect any clinical study of this kind, it is difficult as yet to tell whether the effectiveness of the amino-acids is fully equal to that of casein or dried milk.

These studies with amino-acids point the way to a firmer basis for the definition of protein requirements. In general, when protein is derived from natural foods, if the essential amino-acid requirements are covered, the total nitrogen supply will also be adequate. "Look after the essentials and the total will look after itself." Therefore if the need for each essential is known, the requirement in terms of any protein can be calculated from its amino-acid composition. In this approach it is often implicitly assumed that there is no such thing as giving too much; in other words, that anything above adequate is optimal. *Elvehjem's* work shows that in certain circumstances this assumption is not justified. He found that, in rats on a deficient diet, increasing the amount of one amino-acid may cause an impairment of growth and an increase in liver fat. For instance, on a low-protein diet the addition of leucine has a growth-depressing effect, which is counteracted by isoleucine. The leucine/isoleucine ratio is therefore critical. This work began by a study of the growth of rats on maize diets, maize proteins being notoriously unbalanced in their amino-acid pattern. The warning is obvious: if on a vegetable diet the desired intake is calculated from the amino-acid composition of the proteins, and if this intake is set at a high level in order to provide adequate amounts of the limiting amino-acid (usually tryptophan, methionine or lysine), then other amino-acids will be in excess, and the result may be harmful. However, the principle to be applied in practical nutrition remains the same: safety lies in a mixed diet. But whereas in the past emphasis has always been laid on what may be lacking in an unbalanced protein, this work of Elvehjem's draws attention to the effects of the amino-acids that may be present in excess.

Elvehjem's experiments do not answer the question of whether there is any harm in giving excess of a *balanced* protein. In general, however, he believes that there is nothing gained by increasing the intake above the minimal adequate level. In his view, the principle of determining the minimal requirement, and then doubling it to be on the safe side, is bad. If this is done, 99 per cent of people may be given an excess which might be harmful, in order to meet the needs of the one exceptional subject whose requirements are unusually high. Such a policy represents a mistaken calculation of risks.

If this criticism of "doubling to be on the safe side" is valid, it becomes more important than ever to decide what is meant by the word "adequate", since the zone between too little and too much is narrowed. In children the usual criterion of adequacy is *growth*, by which is commonly meant gain in weight. This crude criterion was criticized by *Gopalan*, on the ground that a gain in weight may represent accumulation of water or fat, and not a true increase in

tissue. Apart from this, the question was raised by *Gyorgy*, whether a maximal rate of weight gain or tissue formation is necessarily in the long run the best for the organism. From the absence of discussion it would seem that there is not much evidence, one way or the other, on this important point.

The same problem arises when the results of work in animal husbandry are applied to man. *Catron* described the exceedingly efficient feeding methods that have been developed for the raising of pigs. After the first few weeks of life, very good performance can be achieved with a purely artificial ration, in which the soya bean is the protein-source of choice. About 20 per cent of the calories must be supplied by protein, and the ration must be properly balanced in respect of vitamins and minerals. The source of carbohydrate is also of some importance. With improved sanitation antibiotics have a negligible growth-promoting effect.

As *Terroine* remarked, we have far more information about the nutritional needs of young pigs than of young human beings, presumably because of their greater economic importance. *Mayer*, however, made the point that "performance" does not necessarily mean the same thing for the two species. In the pig performance is judged by the amount of lean meat put on in a given time or for a given cost—a criterion that can hardly be applied to man, even to the growing infant.

In adults the definition of requirement is even more difficult. *Terroine* emphasized the distinction between the *physiological requirement*—the minimum amount of protein that will maintain nitrogen balance—and the *hygienic requirement*—the amount needed under the stresses of everyday life. The former can be defined with precision, as was done by Rose. The latter is at present a matter of guess-work. *Allison's* experiments throw some light on this problem. In dogs balance can be maintained over a wide range of nitrogen intakes. In the dog on a minimal intake the protein stores are much lower than in the animal fed at a higher level. The term "protein stores" is a vague one, and does not refer to any well-defined anatomical entity, but, as a great deal of modern work suggests, it is none the less a reality. *Waterlow* suggested that the level of the protein stores may be important in determining the rate of interchange of nitrogen between different organs. If this interchange is reduced, the organs with a rapid protein turnover may find their supplies reduced, even though the overall intake for the body as a whole is enough to maintain nitrogen balance.

One result of a decrease in protein stores is a reduction in the weight of some organs, such as liver and pancreas. In the liver *Allison* has shown that there is a fall in the activity of some enzyme systems. Can such an animal with depleted stores be said to be normal? Probably not, since preliminary experiments show that it has a reduced resistance to certain toxic agents. Clearly, such experiments, in which an attempt is made to determine the physiological significance of the level of the protein stores, are of the greatest importance. Their results may provide a rational basis for the concept of a "margin of safety", and so make it possible in the future to define with more precision Terroine's "hygienic requirement".

The salient features of the discussion may be summarized as follows:

1. Experiments on adults and infants with synthetic diets have approximately defined the human requirements for the essential amino-acids. Deductions must be cautious, because of the limited duration and artificial conditions of the experiments. Quantitatively the results on the whole agree with those obtained by feeding whole proteins.

2. The problem of the quality of proteins may be reformulated as the problem of amino-acid balance or imbalance. The new concept is that excess may be as harmful as deficiency.

3. There is an intimate connection between the dietary intake and the level of the body's protein stores or reserves. It is impossible to define either an adequate or an optimal intake until more is known about the functions of these stores, and the conditions under which these reserves are drawn on—in other words, about the relation between protein stores and performance.

References

1. Terroine, E. F. (1956). Les besoins protéiques de l'homme et les conditions de leur satisfaction. *Bull. méd. I.O.F.* In the press.
2. Davis, T. R. A. and Mayer, J. (1955). Protein requirements. Working paper presented to the Princeton Conference on behalf of FAO.
3. Food and Nutrition Board (1953). Recommended Dietary Allowances, Revised 1953. Publication No. 302. National Academy of Sciences, National Research Council, Washington, D.C.
4. Hegsted, D. M., Tsongas, A. G., Abbott, D. B. and Stare, F. J. (1946). Protein requirements of adults. *J. Lab. clin. Med.*, **31**, 261.
5. Rose, W. C., Johnson, J. E. and Haines, W. J. (1950). The amino acid requirements of man. I. The role of valine and methionine. *J. biol. Chem.*, **182**, 541.
6. Rose, W. C., Haines, W. J., Warner, D. T. and Johnson, J. E. (1951). The amino acid requirements of man. II. The role of threonine and histidine. *J. biol. Chem.*, **188**, 49.
7. Rose, W. C., Haines, W. J. and Warner, D. T. (1951). The amino acid requirements of man. III. The role of isoleucine: additional evidence concerning histidine. *J. biol. Chem.*, **193**, 605.
8. Rose, W. C., Warner, D. T. and Haines, W. J. (1951). The amino acid requirements of man. IV. The role of leucine and phenylalanine. *J. biol. Chem.*, **193**, 613.
9. Rose, W. C., Haines, W. J. and Warner, D. T. (1954). The amino acid requirements of man. V. The role of lysine, arginine, and tryptophan. *J. biol. Chem.*, **206**, 421.
10. Rose, W. C., Coon, M. J. and Lambert, G. F. (1954). The amino acid requirements of man. VI. The role of the caloric intake. *J. biol. Chem.*, **210**, 331.
11. Rose, W. C., Lambert, G. F. and Coon, M. J. (1954). The amino acid requirements of man. VII. General procedures; the tryptophan requirement. *J. biol. Chem.*, **211**, 815.
12. Rose, W. C., Coon, M. J., Lambert, G. F. and Howe, E. E. (1955). The amino acid requirements of man. VIII. The metabolic availability of the optical isomers of acetyltryptophan. *J. biol. Chem.*, **212**, 201.
13. Rose, W. C., Leach, B. E., Coon, M. J. and Lambert, G. F. (1955). The amino acid requirements of man. IX. The phenylalanine requirement. *J. biol. Chem.*, **213**, 913.
14. Rose, W. C., Borman, A., Coon, M. J. and Lambert, G. F. (1955). The amino acid requirements of man. X. The lysine requirement. *J. biol. Chem.*, **214**, 579.
15. Rose, W. C., Coon, M. J., Lockhart, H. B. and Lambert, G. F. (1955). The amino acid requirements of man. XI. The threonine and methionine requirements. *J. biol. Chem.*, **215**, 101.
16. Rose, W. C., Eades, C. H., Jr. and Coon, M. J. (1955). The amino acid requirements of man. XII. The leucine and isoleucine requirements. *J. biol. Chem.*, **216**, 225.
17. Rose, W. C. and Wixom, R. L. (1955). The amino acid requirements of man. XIII. The sparing effect of cystine on the methionine requirement. *J. biol. Chem.*, **216**, 763.
18. Rose, W. C. and Wixom, R. L. (1955). The amino acid requirements of man. XIV. The sparing effect of tyrosine on the phenylalanine requirement. *J. biol. Chem.*, **217**, 95.
19. Rose, W. C., Wixom, R. L., Lockhart, H. B. and Lambert, G. F. (1955). The amino acid requirements of man. XV. The valine requirement; summary and final observations. *J. biol. Chem.*, **217**, 987.
20. Albanese, A. A. (1953). Effect of a lysine-poor diet on the composition of human plasma proteins. *J. biol. Chem.*, **200**, 787.
21. Behar, A., Viteri, M. and Viteri, F. (1955). Analisis evolutivo de veintidos casos de Sindrome de Pluricarencia Infantil tratados a base de proteinas de leche. *Rev. Col. méd., Guatemala*, **6**, 48.
22. Ohlson, M. A., Brewer, W., Kereluk, D., Wagoner, A. and Cederquist, D. (1955). Weight control through nutritionally adequate diets. Chapter 15 in Weight Control—A Collection of Papers presented at the Weight Control Colloquium, Iowa State College. The Iowa State College Press, Ames, Iowa, U.S.A.
23. Bricker, M. L., Shively, R. F., Smith, J. M., Mitchell, H. H. and Hamilton, T. S. (1949). The protein requirements of college women on high cereal diets with observations on the adequacy of short balance periods. *J. Nutr.*, **37**, 163.
24. Murlin, J. R., Edwards, L. E., Hawley, E. F. and Clark, L. C. (1946). Biological value of proteins in relation to essential amino-acids which they contain: endogenous nitrogen of man. *J. Nutr.*, **31**, 533.
25. Williams, R. J. (1950). Concept of genetotrophic disease. *Nutr. Rev.*, **8**, 257.
26. Williams, R. J., Beerstecher, E., Jr. and Berry, L. J. (1950). Concept of genetotrophic disease. *Lancet*, **1**, 287.
27. Rose, W. C. and Wixom, R. L. (1955). The amino acid requirements of man. XVI. The role of the nitrogen intake. *J. biol. Chem.*, **217**, 997.
28. Terroine, E. F., Bonnet, R., Chotin, R. and Mourot, G. (1930). Le rôle des sels ammoniacaux organiques, des albumines déficientes et des albumines efficaces dans la couverture de la dépense azotée endogéne spécifique. *Arch. int. Physiol.*, **33**, 60.

[29] Siven, V. O. (1900). Ueber das Stickstoffgleichgewicht beim erwachsenen Menschen. *Skand. Arch. Physiol.*, **10**, 91.

[30] Macy, I. G., Kelly, H. J. and Sloan, R. E. (1953). The composition of milks. *Bull. nat. Res. Coun., Wash.*, No. 254.

[31] Cheung, M. W., Pratt, E. L. and Fowler, D. I. (1953). Total amino-acid composition of mature human milk. *Pediatrics, Springfield*, **12**, 353.

[32] Pratt, E. L., Snyderman, S. E., Cheung, M. W., Norton, P., Holt, L. E., Jr., Hansen, A. E. and Panos, T. C. (1955). The threonine requirement of the normal infant. *J. Nutr.*, **56**, 231.

[33] Snyderman, S. E., Pratt, E. L., Cheung, M. W., Norton, P., Holt, L. E., Jr., Hansen, A. E. and Panos, T. C. (1955). The phenylalanine requirement of the normal infant. *J. Nutr.*, **56**, 253.

[34] Albanese, A. A. (1950). The protein and amino acid requirements of man, in *Protein and Amino Acid Requirements of Mammals*, p. 127, Academic Press, New York.

[35] Beach, E. F., Bernstein, S. S. and Macy, I. G. (1941). Intake of amino-acids by breast-milk-fed infants and amino-acid composition of cow's milk and human milk. *J. Pediat.*, **19**, 190.

[36] Swanson, W. W. (1932). Composition of growth; full-term infant. *Amer. J. Dis. Child.*, **43**, 10. And personal communication.

[37] Albanese, A. A. (1951). Protein and amino-acid requirements of infants. *Pediatrics, Springfield*, **8**, 455.

[38] Albanese, A. A., Holt, L. E. Jr., Irby, V., Snyderman, S. E. and Lein, M. (1947). Tryptophane requirement of the infant. *Johns Hopk. Hosp. Bull.*, **80**, 158.

[39] Albanese, A. A., Holt, L. E. Jr., Davis, V. I., Snyderman, S. E., Lein, M. and Smetak, E. M. (1948). The isoleucine requirement of the infant. *J. Nutr.*, **35**, 177.

[40] Albanese, A. A., Holt, L. E. Jr., Davis, V. I., Snyderman, S. E., Lein, M. and Smetak, E. M. (1949). The sulfur amino-acid requirement of the infant. *J. Nutr.*, **37**, 511.

[41] Scrimshaw, N. S., Guzmán, M. and Mendez de la Vega, J. (1951). The interpretation of human serum protein values in Central America and Panama. *Amer. J. trop. Med.*, **31**, 163.

[42] Leverton, R. M., Johnson, N., Ellison, J., Skellenger, M., Geschwender, D. and Schmidt, F. (1955). Amino-acid requirement of young women: IV. Phenylalanine. *Fed. Proc.*, **14**, 441.

[43] Axelrod, A. E., Beaton, J. R., Cannon, P. R., Chow, B. F., Frazier, L. E., Gaebler, O. H., Hughes, R. H., Leverton, R. M., Pollack, H. and Pruzansky, J. (1954). Symposium on Protein Metabolism. Proceedings of the Nutrition Symposium held at the University of Toronto, Toronto, Ontario, Canada, 1953. Nutrition Symposium Series No. 8. National Vitamin Foundation, Inc., New York.

[44] Jones, E. M., Baumann, C. A. and Reynolds, M. S. (1955). Methionine and lysine requirements of mature women. *Fed. Proc.*, **14**, 438.

[45] Langstein, L. and Edelstein, F. (1917). Die chemische Zusammensetzung frühgeborener Säuglinge und ihr Wachstumsansatz. *Z. Kinderheilk.*, **15**, 49.

[46] Edelstein, F. and Langstein, L. (1919). Das Eiweissproblem im Säuglingsalter. Experimentelle Untersuchungen über die Wertigkeit der Milcheiweisskörper für das Wachstum. *Z. Kinderheilk.*, **20**, 112.

[47] Kaye, R., Caughey, R. H. and McCrory, W. W. (1954). Effects of vitamin B_{12} and aureomycin on nitrogen retention in infants. *Pediatrics, Springfield*, **13**, 462.

[48] Soupart, P., Moore, S. and Bigwood, E. J. (1954). Amino-acid composition of human milk. *J. biol. Chem.*, **206**, 699.

[49] Mellander, O. (1947). On chemical and nutritional differences between casein from human and from cow's milk. *Uppsala LäkFören. Förh.*, **52**, 107.

[50] Mellander, O. (1950). Physiological importance of casein phosphopeptide calcium salts peroral calcium dosage of infants. Some aspects of pathogenesis of rickets. *Uppsal LäkFören. Förh.*, **55**, 247.

[51] Mellander, O. and Isaksson, B. (1950). Physiological importance of casein phosphopeptide calcium salts; intravenous and peroral calcium dosage in animal experiments. *Uppsal LäkFören. Förh.*, **55**, 239.

[52] Woolley, D. W. (1946). Some correlations of growth-promoting powers of proteins with their strepogenin content. *J. biol. Chem.*, **162**, 383.

[53] Woolley, D. W. (1948). Strepogenin activity of derivatives of glutamic acid. *J. biol. Chem.* **172**, 71.

[54] Sprinson, D. B. and Rittenberg, D. (1949). The rate of utilization of ammonia for protein synthesis. *J. biol. Chem.*, **180**, 707.

[55] Aykroyd, W. R. and Swaminathan, M. (1940). Nicotinic-acid content of cereals and pellagra. *Indian J. med. Res.*, **27**, 667.

[56] Squibb, R. L., Braham, J. E., Arroyare, G. and Scrimshaw, N. S. (1955). Supplementatio of low-tryptophan-niacin-deficient diets with beans and lime-treated corn in rats. *F Proc.*, **14**, 451.

[57] Waterlow, J. C. (1948). Fatty liver disease in infants in the British West Indies. *Spec. R Ser. med. Res. Coun., Lond.*, No. 263. H.M. Stationery Office, London.

[58] BROCK, J. F., HANSEN, J. D. L., HOWE, E. E., PRETORIUS, P. J., DAVEL, J. G. A. and HENDRICKSE, R. G. (1955). Kwashiorkor and protein malnutrition. A dietary therapeutic trial. *Lancet*, **2**, 355.
[59] GILLMAN, T. and GILLMAN, J. (1945). Powdered stomach in treatment of fatty liver and other manifestations of infantile pellagra. *Arch. intern. Med.*, **76**, 63.
[60] ALLISON, J. B. (1951). Interpretation of nitrogen balance data. *Fed. Proc.*, **10**, 676.
[61] ALLISON, J. B. (1953). Protein requirements of the diet. *Comptes rendus des Vmes congrès internationaux de médecine tropicale et du paludisme*, **2**, 465.
[62] ALLISON, J. B. (1953). Dietary proteins: their function in health and disease. *J. agri. and food Chem.*, **1**, 71.
[63] HEGSTED, D. M., MOSCOSO, I. and COLLAZOS CH., C. (1952). A study of the minimum calcium requirements of adult men. *J. Nutr.*, **46**, 181.
[64] OLESEN, K., HEILSKOV, N. C. S. and SCHØNHEYDER, F. (1954). The excretion of ^{15}N in urine after administration of ^{15}N-glycine. *Biochim. biophys. Acta*, **15**, 95.
[65] TROWELL, H. C. (1952). Personal communication.
[66] ALLISON, J. B., WANNEMACHER, R. W., Jr. and MIGLIARESE, J. F. (1954). Diet and the metabolism of 2-aminofluorene. *J. Nutr.*, **52**, 415.
[67] MCCOY, J. R., ALLISON, J. B., CROSSLEY, M. L. and WANNEMACHER, R. W., Jr. (1956). Chemotherapy of canine cancer with N-(3-oxapentamethylene)-N′, N″-diethylenephosphoramide (MEPA). *Amer. J. vet. Res.*, **17**, 90.
[68] HAWLEY, E. E., MURLIN, J. R., NASSET, E. S. and SZYMANSKI, T. A. (1948). Biological values of six partially purified proteins. *J. Nutr.*, **36**, 153.
[69] ALLISON, J. B., WANNEMACHER, R. W., HILF, R., MIGLIARESE, J. F. and CROSSLEY, M. L. (1954). Dietary protein and tumor-host relationship in the rat. *J. Nutr.*, **54**, 593.
[70] WAINIO, W. W., EICHEL, B., EICHEL, H. J., PERSON, P., ESTES, F. L. and ALLISON, J. B. (1953). Oxidative enzymes of the liver in protein depletion. *J. Nutr.*, **49**, 465.
[71] WATERLOW, J. C. and PATRICK, S. J. (1954). Enzyme activity in fatty livers in human infants. *Ann. N.Y. Acad. Sci.*, **57**, 750.
[72] ROSENTHAL, H. L. and ALLISON, J. B. (1951). Some effects of caloric intake on nitrogen balance in dogs. *J. Nutr.*, **44**, 423.
[73] ALLISON, J. B. (1949). Biological evaluation of proteins. *Advanc. Protein Chem.*, **5**, 155.
[74] EPPRIGHT, E. S., SIDWELL, V. and JEBE, E. (1955). Food intake and body size of Iowa children. Chapter 9 in Weight Control—A Collection of Papers presented at the Weight Control Colloquium, Iowa State College. The Iowa State College Press, Ames, Iowa, U.S.A.
[75] ALLISON, J. B. (1953). Amino acid requirements of man. *Borden's Rev.*, **14**, 61.
[76] Co-operative determinations of the amino-acid content and of the nutritive value of six selected protein food sources (1951). Bureau of Biological Research, Rutgers University, New Brunswick, N.J.
[77] MITCHELL, H. H. and BEADLES, J. R. (1950). Biological values of six partially purified proteins for the adult albino rat. *J. Nutr.*, **40**, 25.
[78] COPPING, A. M., CROWE, P. J. and POND, V. R. G. (1951). The growth response of rats to purified diets. *Brit. J. Nutr.*, **5**, 68.
[79] BALFOUR, B. M. (1954). Growth, nitrogen balance and histological picture of organs of rats fed on Gambian diets. In *Report of the Second Inter-African Conference on Nutrition, Gambia, 1952.* p. 120. H.M. Stationery Office, London.
[80] PLATT, B. S. (1954). Infant feeding practices; breast feeding and the prevention of infant malnutrition. *Proc. Nutr. Soc.*, **13**, 94.
[81] MURPHY, E. A. and DUNN, M. S. (1949). Influence of environment on preweaning growth of the rat. I. Dietary regimen of the young. *Proc. Soc. exp. Biol., N.Y.*, **71**, 241.
[82] MUNRO, H. N. (1951). Carbohydrate and fat as factors in protein utilization and metabolism. *Physiol. Rev.*, **31**, 449.
[83] BRODY, S. (1945). *Bioenergetics and Growth*. Reinhold Publishing Corpn., New York.

II: HUMAN PROTEIN REQUIREMENTS AT DIFFERENT AGES

Introduction

Aykroyd: We must now turn from experimental work to the practical question of human protein requirements. A conference of this nature cannot, of course, produce an agreed statement on so difficult a problem, but I hope that our discussions may develop in such a way that some general principles may emerge, and that we will be able to see more clearly how the problem can be approached.

In the first session we discussed one aspect of the subject, namely the amino-acid requirements of babies and adults. Dr. Rose himself pointed out that this represents only one of several methods of approach to the problem. It should not be difficult to relate it to other approaches. In our existing knowledge of amino-acid requirements there is a wide gap between infants and adults; that gap, of course, includes the age-group with which we are particularly concerned and which is most prone to suffer from protein malnutrition—the child after weaning. How then, can that gap in knowledge be filled?

I venture to suggest some other approaches to the problem, which turn to a large extent on direct observations on human beings. First, there is the obvious method of observing the kinds of diets which are and are not associated with the presence of protein malnutrition. This may give us some sort of a benchmark or guide to minimum requirements. Secondly, there is the therapeutic approach. People concerned with the treatment of protein malnutrition have very properly been eager to produce the best and most rapid results, and have used large amounts of amino-acid mixtures or of certain types of protein-rich foods. Therefore that particular information, as it stands, may not throw much light on protein requirements from the quantitative standpoint, but it produces valuable information from the qualitative point of view.

There are other observations which may be useful. A great deal of information exists in different parts of the world on the types of diet on which human beings can grow and thrive reasonably well, even if such diets are relativel lacking in protein of animal origin, such as milk. But in making use of suc data we come up against the question: what represents "normal" growth It will be relevant, I think, to consider some of the data available to us on th growth of infants in different countries on different regimes. Another ver important problem is the protein needs of pregnant and nursing women, wit particular emphasis on nursing women.

We also have to face certain practical questions. For example, should protei requirements be stated in grams per kilogram of body weight, as the proportio of calories in the diet to be derived from protein, or as grams of protein pe 100 calories? Some guidance on such points would be useful. We might, o course, think not so much in terms of grams of protein but rather in terms o actual foods; this is related to the amino-acid side of the problem. Therefor we must consider how far available data on amino-acid requirements and on th amino-acid composition of foods can be translated into terms of practic dietetics.

Finally, we must glance again at the question of so-called optimum an minimum requirements in the light of yesterday's discussion, and conside what we mean by these terms in practice. We must hope that these variou

lines may lead us in the direction of a clearer knowledge of protein requirements. At the very least, we must try to define the extent of available knowledge, and the gaps in knowledge which need to be filled.

KING: Dr. Aykroyd has posed a number of questions. I think it may be useful to start by considering his second point—the growth of infants and the diets associated with growth failure. This may be regarded as the initial stage of kwashiorkor, and so leads us on to consider the levels of intake which produce kwashiorkor, and the levels of intake necessary to cure it.

Protein Requirements of Infants and Pre-school Children

SURVEYS IN DIFFERENT REGIONS

In Guatemala

SCRIMSHAW: In many so-called under-developed areas of the world the problem comes not in the first few months, nor during the period in which the mother is feeding the infant, but after weaning. It also does not come in the school-age period. To take Central America as an example, the children do very well in growth and mature well up to the age of about 10 to 14 months, and then bone maturation almost ceases for a period of 3 or 4 years and growth becomes appreciably slower. It is in this period that underlying changes occur, which become apparent as kwashiorkor only with added stress or with further severe restriction in diet.

We wanted to know what food pre-school children were actually getting, who were retarded in growth and maturation and in a state that we refer to as incipient or "pre-kwashiorkor". In the last few weeks INCAP nutritionists have been living during the day in the homes of 40 families from the lower economic segment of a small mestizo town near Guatemala City. The data for 15 of these children are shown in Table 22. To obtain the amounts of animal and vegetable protein, everything that the child ate was weighed and measured. It will be noted that for 2 of the 15 children there was no animal protein at all in the diet. Five of the children in this group ate 2 g. of protein or less per 100 calories. These children may very well be drifting into kwashiorkor in a few weeks or months from now. That we shall know later. These data are presented as an indication of what is actually taking place in many areas of the world.

AUTRET: How many days did your survey take?

SCRIMSHAW: Two days per child.

The degree of retardation in height and weight, compared with well-nourished North American children, is very great. Yet these are not children selected for being ill or for having kwashiorkor or anything else. They are just random children chosen from the lower economic segment of a population in a small town. However, we know from many studies that nearly all the pre-school children in rural population groups in Guatemala and in the poor areas of the city are in a stage of growth failure. Children from these groups dying from causes other than kwashiorkor show mild atrophy of the pancreatic acini; the intestinal wall is usually thin and the rugae atrophic; the liver, although without fat, is below the normal weight for the age of the child. Furthermore, the degree of atrophy of these organs in cases of kwashiorkor at autopsy is greater than would be expected from the relatively short duration of the clinical signs and symptoms. Because of these and other findings, we consider most of the children under 5 years of age from these groups to be in a stage of "pre-kwashiorkor".

TABLE 22

Daily protein and caloric intakes of pre-school children from low-income "mestizo" families, Amatitlan, Guatemala

Subject no.	Sex	Age (months)	Weight (kg.)	Calories	Animal protein (g.)	Total protein (g.)	Protein per 100 cal.	Protein per kg.
1	M.	15	8·5	738	0	24·3	3·3	2·9
2	F.	17	6·1	983	0	19·1	1·9	3·1
3	M.	17	7·2	569	1·2	11·2	2·0	1·6
4	F.	18	10·7	850	11·6	23·2	2·7	2·2
5	F.	20	7·3	683	2·5	10·5	1·5	1·4
6	M.	18	8·5	686	0·3	18·3	2·7	2·1
7	M.	26	9·9	995	6·6	31·1	3·1	3·1
8	F.	26	9·0	1,218	5·1	22·6	1·9	2·5
9	F.	27	8·4	735	10·2	25·1	3·4	3·0
10	M.	30	9·8	1,124	3·7	22·2	2·0	2·3
11	F.	24	12·4	1,509	17·8	48·8	3·2	3·9
12	F.	42	10·5	968	22·4	43·8	4·5	4·2
13	F.	49	15·4	1,825	41·1	58·3	3·2	3·8
14	M.	60	20·3	1,160	3·3	28·9	2·5	3·4
15	M.	62	15·7	1,198	12·7	38·9	3·2	2·5

Averages of two days' consumption from data of the Instituto de Nutrición de Centr América y Panamá and Direccion General de Sanidad Pública, Guatemala, May, 1955.

KING: Do these same children, if properly nourished, tend to conform tc the Western European and American growth curve?

SCRIMSHAW: Unfortunately we haven't very much information yet. We knov that in Guatemala the children for the first 8 to 10 months do follow the Unitec States curve, and we have a few curves of children who were given supplementar cow's milk from the time of weaning. We were trying to prevent the fall in growtl rate, and apparently we are going to succeed. But we haven't enough data ye to demonstrate this in any large number of cases.

An interesting point is that in Central America when the child reaches schoo age and his competitive position in the family improves, his growth and matura- tion tend to parallel those for well-nourished children in the United States he does not gain, but he does not necessarily lose. His potential growth rat during the school years seems to be essentially the same as that of the Nortl American child.

In Dakar

SÉNÉCAL: In Dakar the growth curve of Africans in the first few months o life is above the standard curve for children in Europe (Figure 11). I don' know which you would consider normal. At about 6 months the curve bend and cuts the European curve. Between 12 and 18 months growth goes o slowly or even stops completely. At 18 months the infant is weaned; this is th age at which it crawls about and begins to walk, and so becomes exposed t parasitic infestations. In other words, there are multiple stresses, which some times lead to kwashiorkor. But before developing the typical or complet syndrome many infants pass through a period of "pre-kwashiorkor" or later

kwashiorkor. We are now studying the clinical and biochemical features of this stage. Usually the children are between 12 and 24 months old. When we give them supplementary foods normal growth is restored, and the minor signs of deficiency disappear. If they don't come regularly for treatment their condition deteriorates and some even have to be admitted to hospital with fully-developed kwashiorkor. This deterioration takes place very rapidly—in 2 or 3 weeks.

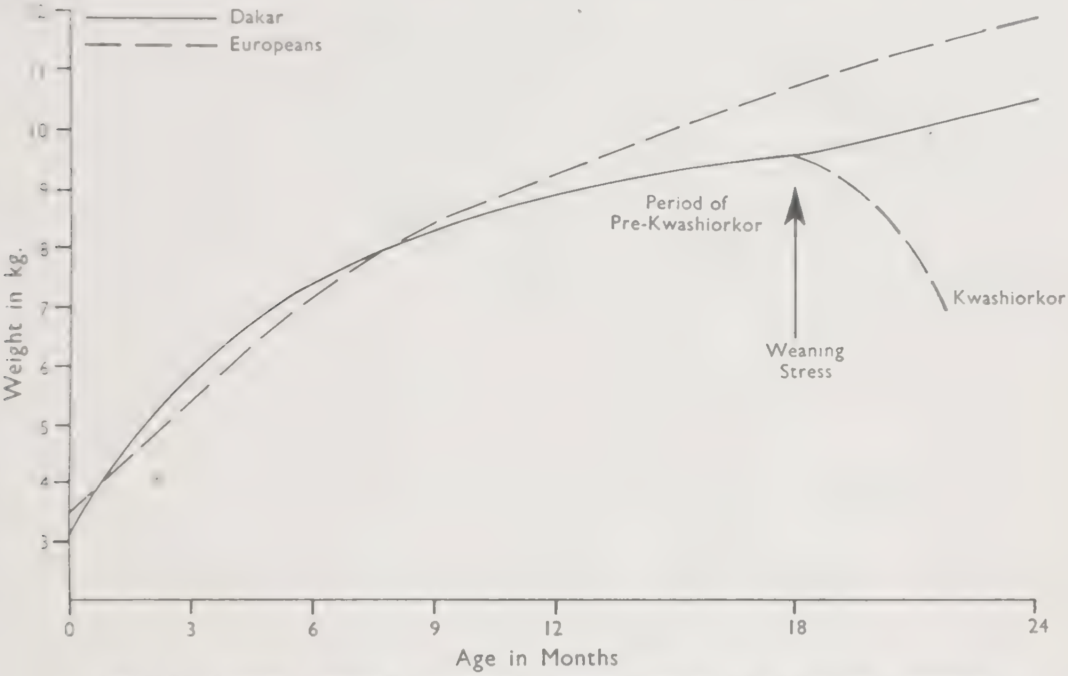

FIG. 11. Growth curve of infants in Dakar (mean of 928 children) compared with European children. Note that the average weight is above the European for the first 6 months of life.

DEAN: There must be many children who do not come to hospital and do not show kwashiorkor but have got kwashiorkor.

FREMONT-SMITH: You mean who actually have protein malnutrition. You can't say they do not have kwashiorkor when they actually have it, but you can say they do not have the clinical syndrome of kwashiorkor but do have protein malnutrition. Or would you speak of kwashiorkor sine kwashiorkor?

DEAN: Yes.

FREMONT-SMITH: Well, I dislike that.

In the Belgian Congo

DE MAEYER: I would like to show a few data on the growth of breast-fed children in the Belgian Congo compared to a group of Belgian children.[1,2] These observations are shown in Figure 12. Curves C and D were obtained by Dr. Holemans in Kwango from two groups of Basuku children. One group was unsupplemented, the other one got a daily supplement of skimmed milk. Curve B has been derived by us from data on Bashi children recorded at a postnatal clinic in Bukavu (Kivu). It is highly probable that this curve after 8 to 9 months is artificially low, only representing the badly fed children who are left attending the clinic. The fourth curve (A) shows the growth of a group of Belgian children.

Thus we have here figures about the growth of children from two differen parts of the Congo. These figures are really different during the first months o life, but they show the same distinct drop at about the same time (7th month). That the difference in the growth curves during the first months is not genetic i shown by the fact that it is possible to reduce or even to suppress it, at least fo a time, just by supplementing the diet. The failure of growth after 7 months which seems to be general, may be caused by lack of adequate mixed or supple mentary feeding in the breast-fed child, and also by the fact that at this time h begins to crawl, and so becomes exposed to all kinds of infestations.

AUTRET: The Basuku are very poor people from the central part of Africa The average weight of the mother is only 38–39 kg. and she is in a very poo nutritional state. The weight of the child at birth is 2·4 kg. On the other hand in other places you have birth weights that are higher than the average Europear figures. It depends on the nutritional status of the mother, the economic leve of the country, and many other determining factors. There are great differences between Dakar, for example, and Gambia, which is close to Dakar. You migh find about twenty different patterns in Africa.

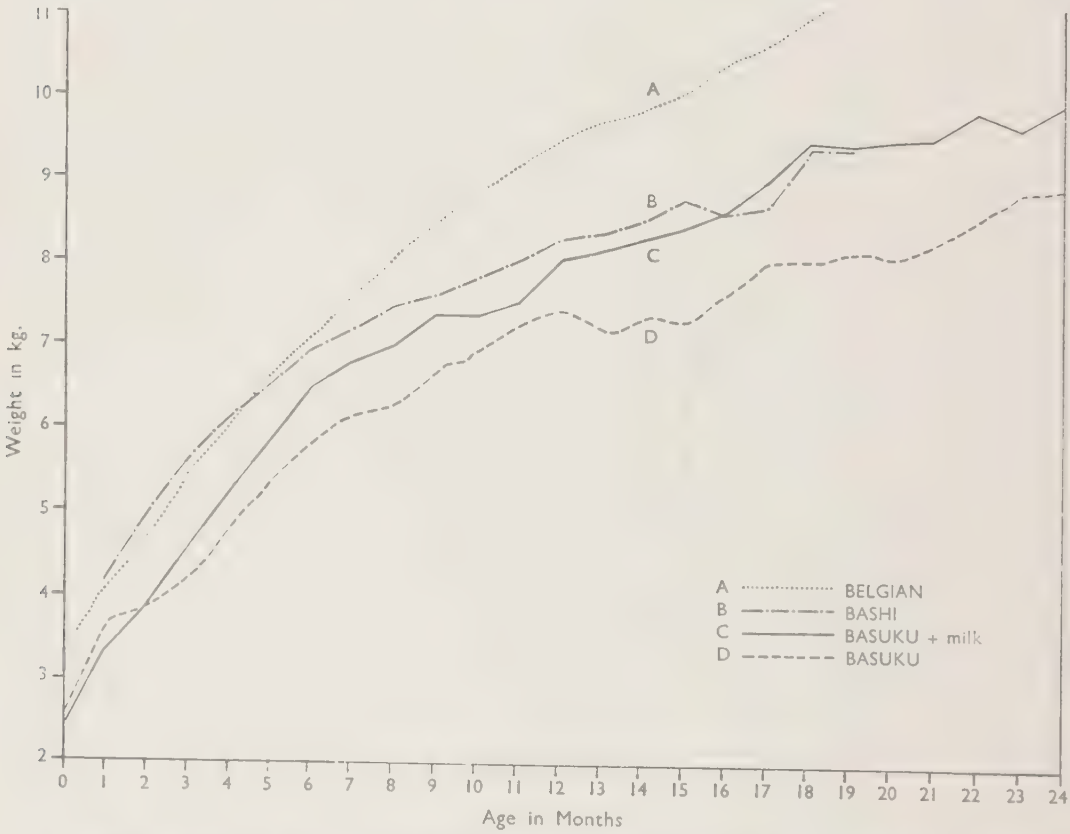

FIG. 12. Growth curves of infants in two districts of the Belgian Congo. B, Bashi children in Bukavu. C and D, Basuku children in Kwango. (Data for C and D from Holemans.[1])

SCRIMSHAW: At what age do you start to find kwashiorkor in the Basuku In Guatemala our growth curve does not begin to fall until 8 to 10 months whereas yours starts dropping at 2 months or even at birth. Therefore you ough to find kwashiorkor occurring at an earlier age than we do in Central Americ where it usually develops at about 2 years.

DE MAEYER: The earliest appearance is at about 12 months among th Basuku (Kwango) and between 24 and 30 months among the Bukavu (Kivu

This difference in the age of onset might be due to the milk supply of the mother: as Dr. Autret pointed out, the Kwango mothers are in a very poor state. Or it might be due to a difference in the supplementary food that the babies get.

PLATT: At what age do the Bukavu stop breast-feeding?

DE MAEYER: 24 months.

DEAN: Is the child not allowed to get at the breast after 24 months?

DE MAEYER: Yes, but there is not much left.

CRUICKSHANK: What are the supplementary foods that these babies get?

DE MAEYER: The Basuku get a supplement consisting mainly of cassava. Among the Bukavu the supplement is a mixture of various kinds of black and white beans.

WATERLOW: We see the same two patterns in the onset of kwashiorkor on our side of the Atlantic. The Basuku have a cassava supplement and an early onset, the Bukavu a bean supplement and a late onset. Similarly, in Guatemala the children get beans, and seem to drift slowly into a state of kwashiorkor. In the West Indies, on the other hand, the supplement sometimes consists of nothing but sugar and starch, and then the baby, once off the breast, goes downhill rapidly.

SCRIMSHAW: In Uganda plantains are used, which are low in protein, and there the onset is also early; isn't that so, Dr. Dean?

DEAN: Yes.

WATERLOW: From the histological sections that I saw at INCAP I got the impression that the amount of fibrosis in liver and pancreas is much greater than we commonly see in Jamaica. This suggests that kwashiorkor in Guatemala is not only late in onset, but becomes much more chronic.

SCRIMSHAW: We have two types of case in Central America. One group drifts slowly into kwashiorkor; in the other an episode of infectious diarrhoea or some febrile illness throws them into kwashiorkor in a matter of 3 or 4 weeks.

DE MAEYER: Among the Bukavu, most cases of kwashiorkor appear after some infectious disease, usually after measles or glandular tuberculosis. With the Basuku the role played by infectious diseases does not seem as important, and most cases go slowly downward into kwashiorkor.

CRUICKSHANK: Is the low growth-rate an indication of protein malnutrition or of calorie deficiency? What is the calorie intake in the critical period?

DE MAEYER: In both Kwango and Kivu the calorie intake seems adequate.

CRUICKSHANK: What was the protein content of the diet for the Bukavu children who got the beans?

DE MAEYER: They get a fairly large amount of beans, but I cannot give you exact figures. I have, however, some data for Basuku children recorded by Dr. Holemans.[2] These are the children with the lowest growth curve (D) in Figure 12. The intakes were measured while the children were in hospital, and are shown in Table 23. The milk intake was measured by weighing the children before and after feeding. The mothers were also asked to bring to the hospital the food they used to give as a supplement. This was always a paste of cassava (luku) which contained about 45 per cent of starch. The children did not receive anything else. Analysis of the data shows that the calorie intake of these infants is fair but that the protein intake is low.

AUTRET: I want to discuss the curves in Figure 12 in more detail, and to add some data on the growth of older children, from 2 to 5 years (Figure 13). These were obtained during a pilot supplementary feeding programme organized by the government of the Belgian Congo in an attempt to do something about

kwashiorkor in the Kwango. We intended to find out first of all: (1) was it economical to try to distribute milk in the difficult conditions of the Central African bush? (2) were we going to reduce the incidence of kwashiorkor? and (3) were we going to see any beneficial effect on the children's health in general? It is the first time, I think, that such observations have been made under the real conditions of life in Africa.

TABLE 23

Daily food intake of Basuku children

Age (months)	Milk (g.)	Luku (g.)	Carbohydrates (g.)	Protein (g.)	Lipids (g.)	Calories
0–1	350	113	80	4·4	12·2	450
1–2	436	96	79	5·5	15·3	477
2–3	405	108	82	5·1	14·2	476
3–4	380	206	126	4·8	13·3	644
4–5	417	207	129	5·3	14·6	667
5–6	415	223	137	5·2	14·5	699
6–7	323	232	134	3·6	11·9	659
7–8	293	202	117	3·2	10·7	570
8–9	293	194	114	3·2	10·7	564

FIG. 13. Growth curves of older children in the Kwango district of the Belgian Congo. The figure shows the effect of supplementary feeding with skimmed milk.

The distribution of skimmed milk was made on the following basis, according to the recommendations of FAO and WHO:—

40 g. dried skimmed milk a day (250 c.c.) to the nursing mothers, until the baby was 1 year old;

20 g. dried skimmed milk a day (125 c.c.) to the child between 1 and 2 years of age;

40 g. dried skimmed milk a day (250 c.c.) to the child over 2 years of age.

These quantities were adopted not because they were found sufficient, but because they are the amounts that can be drunk at one time after reconstitution into liquid form. Both the supplemented and the control groups (Figure 12, C and D) included about a thousand children.

Although the growth of the control group (D) is as good as that of the supplemented group (C) until the child is 1 month old, there is then a slowing down in the control group. The difference between this curve (D) and the Belgian standard curve (A) is more than 1 kg. at 6 months, 2 kg. at 12 months, and almost 3 kg. at 2 years. In the supplemented group (C), the growth curve shows a fairly satisfactory rise, improving as each new supplement is introduced. When the child reaches 3 years there is a little slowing down of the curve, which however remains satisfactory (Figure 13).

What conclusion may we draw from this study? First, perfectly satisfactory growth until 6 or 7 months is possible in children breast-fed by a mother in a poor nutritional state when the mother is given a dietary supplement, in this instance milk. Would satisfactory growth have been maintained longer if we had given the mother more dried skimmed milk, let us say 80 g.? Probably the answer is " yes". The growth curve improved when the child was given the milk directly at 12 months. Could he receive it directly when he is, let us say, 8 or 9 months old? The answer is probably " yes ", but if this is to be done the mother must be able to give the milk to the child at home under good sanitary conditions. The small quantity of skimmed milk the child received after 1 year of age (20 g.), plus some breast milk, was enough to ensure a satisfactory growth curve up to 18 months.

Another point which Dr. Holemans has stressed[3] is the significant increase in the output of breast milk when undernourished mothers were given additional skimmed milk. The output of milk by mothers in the control group was on an average 300 g. a day when the child was between 1 and 2 months, and 400-500 g. at 5 months; from then onwards it went down to 350 g. and less. The mothers who got skimmed milk during the last 3 months of pregnancy and while they were nursing the baby produced 550 g. a day between 1 and 2 months, 440 g. a day between 2 and 3 months, 450 g. between 3 and 4 months, and then about 430 g. a day until 6 months. Not only was the output of milk increased, but its protein content increased from 1·0 g. to 1·2 g. per cent. This is an important observation, which needs confirmation however, since this is the first time it has been shown that supplementary feeding influences the protein content of the milk.

PLATT: I think the increase in output is more significant than the 0·2 per cent increase in protein content.

KING: How many children were there in the study?

AUTRET: In the growth study there was a total of 1,100—between 70 and 100 for each month of age. The number of mothers whose milk was measured was 62 in the control group and 27 in the supplemented group.

SCRIMSHAW: In Central America it has been recommended that the pre-school child of 2 to 5 should be given 40 g. of skimmed milk a day. This is

because 20 g. once a day doesn't seem to be enough, although our evidence is not as good as Dr. De Maeyer's.

VERHOESTRAETE: These observations are very interesting, but they refer to an area which is rather exceptional in terms of malnutrition—one of the worst in the world. Therefore we should make it clear that *in this particular case* the milk supplement was insufficient, in terms of the specific diet of those women.

I would like to ask Dr. De Maeyer if he has any idea how much, or rather, how little protein these women were receiving in their diet, in order to produce this pattern of lactation and this kind of growth curve.

DE MAEYER: It is difficult to say. It must be very low, I guess no more than 20 g. a day.

DEAN: To what extent can we be certain that this is a protein effect? We know that these people are extremely undernourished, and we know that any addition to their diet will almost certainly make an improvement. When we give dried skimmed milk, we give one and a half times more calories from lactose than calories from protein. Isn't it possible that some of this effect is due to an increase in calories, if the people are on such an extremely low-calorie diet to begin with?*

DE MAEYER: I think that the imbalance in the Basuku diet is very important. If we go back to the growth curve and the food intake of the Basuku children (Figure 12 and Table 23), we see that although the caloric intake is fair, the protein content of the diet is low. The addition of 20 g. of skimmed milk powder (70 calories—7 g. of protein) strikingly increases the growth curve; it is difficult to believe that a 10–15 per cent increase of an already normal caloric intake will make this difference. On the other hand the protein intake, which was initially low, is more than doubled by the addition of the skimmed milk.

In India

GOPALAN: I think that Dr. Aykroyd pointed out, very rightly, that in formulating the minimum requirement, it would be useful to have information on the actual protein intakes in these undernourished communities. At present we know very little about the intakes of young children between 6 months and 5 years, and of lactating women. WHO has financed a project for the prevention of protein malnutrition in India, and as a preliminary step in this campaign we have just begun a survey of protein nutritional status in some southern Indian villages. I have here some preliminary data from some 450 children between 6 months and 5 years old.

Table 24 shows the average total protein intake and calorie intake in these children, and Table 25 shows the food sources from which the protein is derived. It will be seen that in the age group from 6 months to 1 year the protein intake was 11·4 g. daily, and the greater part was derived from breast milk. The interesting point is that between the first and second years, when the total protein intake was about 13 g. daily, a large part of that was derived from breast milk also. The protein intake for the higher age groups was about 22 or 23 g.; a great proportion of this protein came from cereals and a smaller proportion from pulses. They got very little, if any, protein from animal sources.

* Gunther and Stanier[4] in their studies on lactating women in Germany in 1946–9 found that extra fat or extra calories in the form of bread and sugar did not significantly alter the percentage of fat or protein in the milk secreted on the eighth day of lactation. (Editor.)

TABLE 24

Protein and calorie intakes of poor-class infants and pre-school children in South India

Age (years)	Height (in.)	Weight (lb.)	Average calorie intake per day	Average total protein intake per day (g.)
½–1	24·5	13	550 (480–604)	11·4 (7·2–21·9)
1–2	29·0	18	498 (277–951)	12·8 (6·7–22·5)
2–3	31·0	21	585 (177–1,409)	15·2 (6·5–33·8)
3–4			753 (264–1,405)	19·4 (5·8–40·0)
4–5	36·0	27	899 (336–1,800)	23·3 (8·6–44·8)
5			900 (300–1,357)	21·9 (10–33·1)

Figures in brackets indicate range.

TABLE 25

Sources of protein in the diets of poor-class infants and pre-school children in South India

Average protein intake in g. per day from the sources indicated.

Age (years)	Cereals (g.)	Pulses (g.)	Meat (g.)	Breast milk (g.)	Cow's milk (g.)	Total (g.)
½–1	0·74 (0–2·7)	—	—	7·1 (6·9–7·2)	0·54 (0–2·7)	11·4 (7·2–21·9)
1–2	4·3 (0·9–16·8)	2·4 (0–9·4)	0·13 (0–2·5)	3·35 (0–5·7)	2·63 (0–10·8)	12·8 (6·7–22·5)
2–3	8·9 (0–23·1)	3·0 (0–15·7)	0·9 (0–10·6)	1·2 (0–5·4)	1·2 (0–8·8)	15·2 (6·5–33·8)
3–4	11·8 (3–28·2)	3·5 (0–12·6)	1·2 (0–5·3)	—	2·3 (0–15)	19·4 (5·8–40·0)
4–5	15·0 (4·6–30·7)	4·0 (0–12·6)	2·7 (0–10·6)	—	1·3 (0–9)	23·3 (8·6–44·8)
5	15·47 (10–25)	4·4 (0–12·6)	1·3 (0–5·3)	—	0·83 (0–7)	21·9 (10–33·1)

Figures in brackets indicate range.

The position with regard to protein nutrition in these children is much worse, I should say, than is indicated by the figures for protein intake taken by themselves. We have to take into account other factors as well as the actual protein intake. It has often been our experience, and I am sure that other workers in under-nourished countries can bear me out, that when the protein intake in a diet is low, the diet is also deficient in many other things—for example, in vitamins

of the B complex, which may well have an aggravating effect. Then it is invariably found that in communities existing on low levels of protein, there is a corresponding deterioration in the environment; there is always the possibility of infections and infestations adding to the effects of protein deficiency.

I think these points must be remembered in formulating the optimum requirements for protein in undernourished countries, as opposed to the minimum requirements. There is no doubt that all these children whom we investigated suffered from protein malnutrition, and I speak of protein malnutrition advisedly, not using the word "kwashiorkor". I should emphasize here that it would be wrong to speak of protein malnutrition and kwashiorkor as though they were synonymous. If we are going to compute the incidence of protein malnutrition purely by the incidence of kwashiorkor in the community, I think we would run into the danger of considerably minimizing the tremendous magnitude of the problem.

All these children suffered from protein malnutrition but they did not have kwashiorkor, as we understand it. They all had haemoglobin levels ranging from about 8 to 9 g. per 100 ml. In some cases the total serum protein level was well below 6 g. per 100 ml. They all were stunted in growth. Quite a few had discoloration of the hair and enlargement of the liver. Some had skin changes. But we deliberately excluded from this group children who actually presented the full-fledged picture of what we call the nutritional oedema syndrome or "kwashiorkor".

WATERLOW: The calories are also low.

GOPALAN: Yes. We have to take that into account also.

Table 26 shows the 24-hour breast milk output in a series of mothers belonging to the poorer classes, in which the incidence of kwashiorkor is high. The table also shows the protein and caloric intake of these mothers. Perhaps we may discuss this later when we come to consider lactation. It is interesting that the lactation performance was so good, particularly in the later stages, in spite of the relatively poor diets on which these mothers were existing. Of course, there was a wide scatter in the figures. We have had some mothers beyond the 24-month-period also, and were surprised to find that in some of them the 24-hour milk yield was sometimes as high as 30 oz.

TABLE 26

Milk yield and protein and calorie intakes during lactation

Data from 98 nursing mothers of the poorer class in South India.

Period of lactation (months)	Calories per day	Protein (g. per day)	Milk yield (oz. per 24 hr.)
0–6	2,471	57·4	21
6–12	2,139	56·3	19
12–18	1,708	47·2	16
18–24	1,383	44·4	12

Table 27 shows the results of analysis of breast milk in some of these mothers. It is clear that the composition of the milk from these undernourished women subsisting on borderline diets compares quite well with the published figures from Europe and America.

TABLE 27

*Percentage composition of human milk of poor mothers in South India**

Subjects	No. of samples	Protein	Lactose	Fat	Water	Total solid g. per cent
Indian women apparently healthy (Nilgiris)	20	1·19	6·91	4·11	87·97	12·03
Range		0·90–1·91	6·0–7·46	1·84–8·30	85·63–89·0	11·00–14·37
Mothers of children suffering from nutritional oedema syndrome	9	1·24	7·3	4·44	87·24	12·76
Range		0·99–1·46	6·72–8·34	2·04–8·31	85·47–88·96	11·04–14·53
European mothers[5]		1·2	6·5	3·6	87·97	12·03
African mothers[6] ..		1·04		4·7		
Range		0·59–1·76		1·1–9·72		

* From Dr. M. K. Ramanathan, Nutrition Research Laboratories, Coonoor (unpublished data).

Two years ago in Jamaica I made the rather rash pronouncement that the habit prevalent in some countries of feeding children at the breast for prolonged periods, for two or two and a half years, may not have much nutritional significance. Now I think I should retract that statement. (Cries of "Hear, hear!") It does seem that breast milk makes a significant contribution in our children, even in the 18- to 24-month period. The degree of protein malnutrition would be much worse but for that. I think there is much to be said in favour of the suggestion thrown out by Dr. Autret that a very important method of preventing protein malnutrition in children would be to augment the milk supply or the milk yield of the mothers.

One of the other factors to be taken into account in formulating the optimum protein requirements in these communities is roundworm infestation. By this I do not mean 6 or 8 worms, but 100 or 150. We always have to qualify by stating how heavy the worm-load is. Not everyone would agree with this. We have, however, done some experiments on pups which seem to show that heavy roundworm infestation itself is probably an effect of protein malnutrition. It is impossible to infest pups if they get adequate amounts of protein in the diet. Secondly, we have found that in malnourished children there is a considerable difference in the absorption of protein before and after disinfestation. In nine children the average faecal nitrogen output was 19 per cent of the intake before, and 10 per cent after disinfestation, the intake being kept the same.[7]

DARBY: To return to your point about improving lactation performance: for how long do you think that human milk is optimal for the human infant?

GYÖRGY: Dr. Dean has stated,[8] and Dr. Mayer also in his review,[9] that only up to 6 months can breast milk be considered physiological food.

DEAN: I said that from records of lactation it was rather unlikely that nature intended the supply of milk provided by the mother to be entirely adequate without supplementation for longer than 6 months.

GYORGY: But you cannot say that if she produced enough, it would not be satisfactory.

DEAN: No, we cannot say that.

SCRIMSHAW: In Central America the quantity of milk drops off very rapidly toward the end of the first year, and even though the child may be pulling at the breast at 24 months, it is doubtful if he gets any significant nutritional contribution from it.

KWASHIORKOR IN BREAST-FED INFANTS

WATERLOW: I suppose the occasional examples that have been recorded of kwashiorkor developing in a child on the breast result simply from the amount of milk being too small.

DEAN: I was at one time doubtful whether the children described by Gelfand[10] in Southern Rhodesia, who developed kwashiorkor while on the breast, could really have been receiving adequate amounts of breast milk. However, when I was with Gelfand he showed me mothers of children suffering from kwashiorkor whose breasts appeared to have plenty of milk in them and from which milk could be expressed very easily, although the children had recently been sucking. He seems to be quite certain that the woman is contributing an apparently satisfactory amount of milk, although there is no possible doubt that the child has kwashiorkor.

SCRIMSHAW: I agree with you. I didn't believe it in Jamaica,[8] but we have since had one very clear-cut case. This child was exclusively breast-fed—as we know from careful enquiry—until, at the age of 18 months, it was admitted to hospital with frank kwashiorkor. I don't have an explanation.

PLATT: I have seen effects of shortage of protein in entirely breast-fed infants at 6 months.[11,12] I believe that the explanation is almost certainly that they have not had *enough* breast milk.

HANSEN: Then the thing we need to know is the volume of breast milk that Dr. Scrimshaw's child got.

SCRIMSHAW: At the time when the mother brought the child to hospital a sample of about 125 c.c. was expressed from one breast. The following day, in a visit to her home, another 125 c.c. was expressed—this time from the other breast. Test feeds, however, were not done. Analysis of the milk showed values within normal limits for protein, calories, methionine, tryptophan and lysine.

DARBY: Might not the explanation be that there is another disease process producing the same clinical picture? This can happen with megaloblastic anaemia. The diagnosis might easily be missed in an area where kwaskiorkor is endemic.

SÉNÉCAL: Another such possibility is fibrocystic disease of the pancreas. At the Jamaica Conference I described two cases which mimicked kwashiorkor.[8]

DEAN: I don't think that explanation will hold for cases that recover.

FREMONT-SMITH: The resistance to accepting the diagnosis of kwashiorkor in the breast-fed infant is rather significant. Surely there are still enough unknown factors for it to be possible that under certain circumstances the clinical syndrome of kwashiorkor, which, I insist, is a clinical syndrome and not yet a specific disease, can occur sometimes without breast milk and sometimes with breast milk; it may be a question of quantity and quality of breast milk.

or there may be other environmental factors which make just the difference between one and the other. A good many other clinical syndromes have gone through the same kind of history. These apparent exceptions open the door, not for rejection or acceptance, but for special studies on what the situation is in them that is different from the others.

SCRIMSHAW: One quite plausible explanation of the case I quoted is that the child who was 18 months old was at a stage when even a reasonable amount of mother's milk was not supplying enough of the essential amino-acids, and there was, in addition, some stress, perhaps from an infectious diarrhoea; the combination was enough to produce an ordinary case of kwashiorkor.

WATERLOW: Besides, this case might have different requirements, just as Dr. Rose's adults had different requirements, for reasons we don't understand. This child might be the one right at the top of the scale.

SCRIMSHAW: Yes, and the fact that it had a higher degree of macrocytosis suggests that it might be a little outside the normal range of variation.

HOLT: You bring up a very interesting question, that stress may be a factor, and I think that there may be stresses creating additional demands for one or another of the amino-acids.

SÉNÉCAL: The stresses are of many kinds. They may be infectious or parasitic or even psychological. In collaboration with Dr. Dean we are now analysing the results of weaning on the development of kwashiorkor, finding out what the children eat and how much when separated from their mothers. We are beginning to think that the shock of separation from the mother may play a part in producing the disease.

SCRIMSHAW: I think this factor of stress is very important. Children will go along on these grossly deficient diets, growing very little, for year after year, yet not develop acute kwashiorkor. Then one of these stress factors is imposed, and in 3 or 4 weeks they come to hospital with all the signs of kwashiorkor. We suspect from the autopsy findings that some of the pathological changes that we find have antedated this period of acute development and must have been taking place during a previous long period of under- and mal-nutrition. The gross evidence of this is that at times the pancreas is so small that it can scarcely be located. The liver is also frequently far below expected weight, and the gastro-intestinal tract sometimes has a wall that looks like China paper—changes which the pathologist can't conceive of as taking place in the relatively short period of time given in the clinical history.

KING: We are assuming that these infants start off normally well-nourished at the time of birth. But is that necessarily true? For instance, a report has been published,[13] and widely cited, of liver injury in newborn infants induced by malnutrition in the mothers.

PLATT: Silvera thought that his findings suggested that maternal malnutrition may have repercussions on the child in the intra-uterine period, and Woodruff[14] also remarks that protein malnutrition may start in intra-uterine life.

WATERLOW: I have seen the sections of the livers described in that report, and I cannot agree with the author's interpretations. The diagnosis of fatty liver by the microscope can be misleading.*

SÉNÉCAL: In Dakar we have examined 300 newborn babies by biopsy of the liver. In most cases the liver was normal. We found only a few cases of fatty

* Dr. O. Lindan kindly determined the fat content of these livers. In 14 cases, one had 4·9 g. fat per 100 g. tissue, another had 13·7 g. fat per 100 g.; the rest were all below 4 g. per 100 g. (J. Waterlow).

liver, and these resembled more the steatosis of anoxia than that of malnutrition. The fat was predominantly centrilobular and not peripheral.

CATRON: At Iowa State College and elsewhere it has been found that you do not get fatty liver in the baby pig, even when the sow is on a diet as low as 9 or 10 per cent of protein, provided that during pregnancy she gets enough vitamins of the B-complex (riboflavin, niacin, pantothenic acid, choline, and B_{12}). Before the importance of the vitamins was realized we often saw fatty liver in baby pigs that died at or shortly after birth.

SCRIMSHAW: At the Jamaica Conference we presented evidence of low serum vitamin levels in newborn infants.[15]

PROTEIN LEVELS AND NITROGEN BALANCE IN THE TREATMENT OF KWASHIORKOR

SÉNÉCAL: Since Dr. Aykroyd brought up the point, I would like to give some of the results of the treatment we are using for kwashiorkor. I am a little hesitant about this, because so far we have been talking about minimum requirements, while in treating kwashiorkor we are studying the maximum or optimal requirements of protein for undernourished children.

AYKROYD: I think it is useful to have this. It gives us one range of figures which will help in due course when we discuss requirements.

SÉNÉCAL: We discussed in Jamaica[8] the maximum amount of protein that could be given to a child suffering from kwashiorkor. Dr. Dean said that he was giving from 110 to 120 g. a day. Some of us protested, saying that that was far too much. Yet it would seem that amounts of this order can be well tolerated. Table 29 shows that between the 8th and 18th days we reach an average intake of 6–9 g. protein per kg.

SCRIMSHAW: Do you find, as we do, that some of the children receiving these large intakes fail to gain weight over a long period of time?

SÉNÉCAL: No. Our cases lose their oedema, and immediately begin to regain weight. This is shown in Figure 14, which represents a typical case.

DEAN: In Uganda we have not often observed this failure to gain weight. In a few cases we were able to make the growth curve start upward again by giving very large doses of vitamin B_{12}.

SÉNÉCAL: We have never given B_{12}, but we do find that our high protein treatment is only effective when we also increase the calorie intake. This we do from the very beginning by the addition of carbohydrate.

TERROINE: Do you know your coefficient of digestion?

SÉNÉCAL: We have made some measurements of nitrogen balance, with two objects: first, to find whether such large intakes of protein were utilized—in other words, to measure nitrogen retention; secondly, to find out whether such a regimen would in the long run lead to toxic effects.

The results are shown in Tables 28 and 29. The balance was always positive. The percentage nitrogen retention was high, and remained high even after 50 days on a very high protein regimen. In answer to Professor Terroine's question, in almost all cases more than 80 per cent of the ingested nitrogen was absorbed. The ratio of urinary to faecal nitrogen was always greater than 1.

DEAN: In my balance studies nitrogen retention at the beginning of treatment was not as high as later on.

SÉNÉCAL: Our figures (Table 28) do not show any definite trend of that kind. Therefore we think that our children assimilate well right from the start: this is probably because they are getting a hydrolysate of protein.

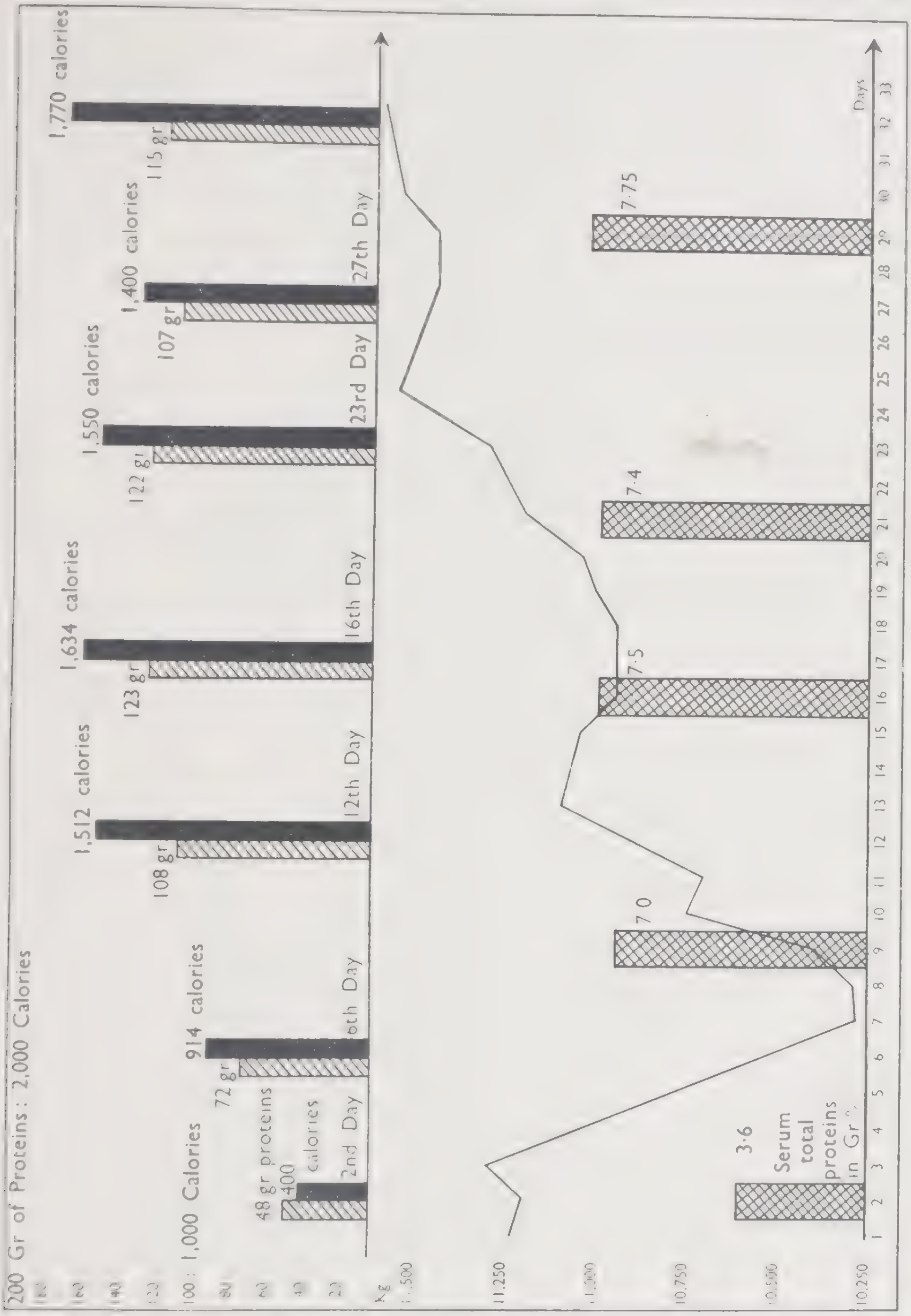

FIG. 14. Data from a typical case of kwashiorkor, successfully treated. The top half of the figure shows the rapidly increasing food intake; the bottom half shows the changes in body weight and serum total proteins.

TABLE 28

Nitrogen balance data during various stages of the treatment of kwashiorkor

Mean values from 6 cases

Days after admission	Number of balance periods	Intake				Nitrogen output per 3 day balance period				
		Total calories	Percentage of calories from protein	Protein (g. per kg. per day)	Nitrogen (g. per 3 days)	Urine (g.)	Stools (g.)	Urine / Stools	Balance (g.)	Percentage retention
1–6	6	262	42·8	3·05	13·0	6·4	2·45	2·7	+ 4·15	35·9
8–18	4	900	30·2	7·25	33·1	12·6	5·65	2·6	+14·85	44·5
30 +	7	967	24·6	5·8	27·5	12·6	3·95	3·65	+11·0	37·0

TABLE 29

Nitrogen balance data from individual cases during recovery phase

8–18th days of treatment with protein hydrolysate, milk, meat, vegetables and fruit.

Case no.	Percentage of calories from protein	Protein intake g. per kg. per day	Nitrogen balance g. per 3 days	Percentage retention
5	25·8	6·62	+23·7	65
2	28·7	7·2	+14·1	50
4	32·4	6·6	+ 8·8	27
3	34·0	8·6	+12·9	37

HANSEN: We also have found that nitrogen retention is the same at the beginning of treatment as at the end. It is remarkable the efficiency with which these cases utilize nitrogen. Figure 15 shows that, on a weight basis, the cases of kwashiorkor retained nearly twice as much nitrogen as the controls. Two of these cases were on a synthetic amino-acid mixture.

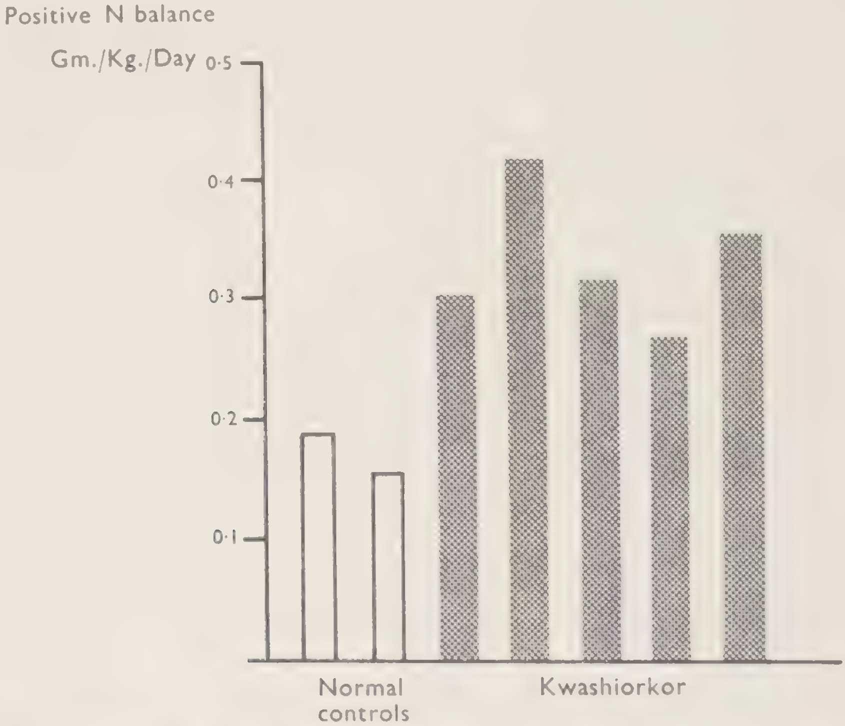

FIG. 15. Nitrogen retention during recovery from kwashiorkor is higher than in normally growing children of the same age. This is evidence of previous depletion of body protein.

SÉNÉCAL: I think also that there may be a relation between the retention and the proportion of calories derived from protein. The optimum retention seems to be when this ratio is between 25 and 30 (Table 29).

TERROINE: This is in agreement with all the studies that have been made of protein utilization in growing animals. There is an optimum relation between the amounts of protein and of carbohydrate in the diet; if you give more protein there is no equivalent increase in the amount of tissue protein formed.

Serum Potassium Levels

SÉNÉCAL: Protein may not be the only limiting factor in recovery. We must take into account other constituents of cellular tissue, and particularly potassium. Our cases on admission show low levels of serum potassium (average 3·5 meq. per litre, rising to 4·7 meq. after 1 week of treatment). They are also in negative

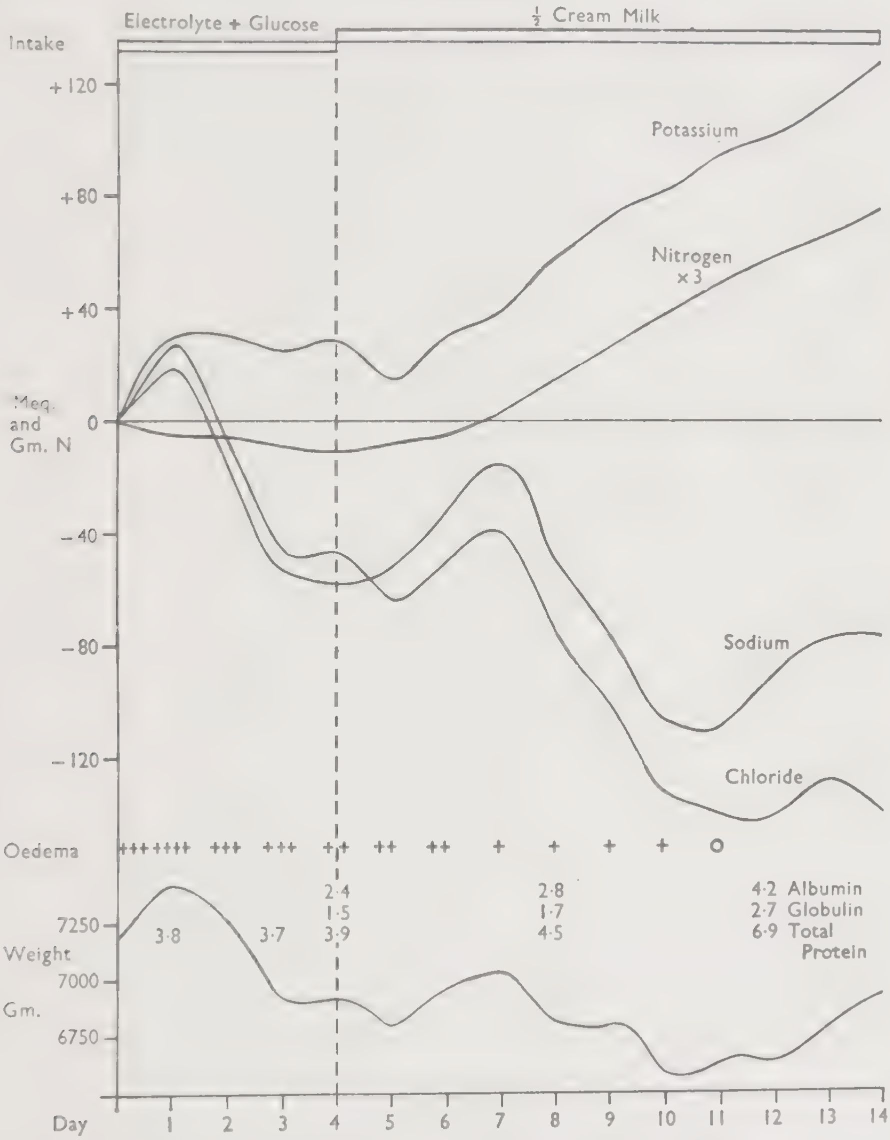

FIG. 16. Cumulative nitrogen and electrolyte balance in a case of kwashiorkor (W.D.) during the first two weeks of treatment.

potassium balance, which is presumably evidence of tissue destruction. Therefore, in order to restore the blood and tissue levels, we give large doses of potassium by mouth (200–400 mg. per day).

HANSEN: In Cape Town we also find low serum potassium levels on admission —below 3·4 meq. per litre. We have done balance studies which show how great the deficit of potassium is.[16] Some of our results are illustrated in Figures 16 and 17. They suggest that potassium deficiency may have something to do with sodium retention and oedema. As potassium is retained there is a sodium

diuresis and loss of weight. This may happen without any change in serum protein levels (Figure 16), and even in the face of a negative nitrogen balance (Figure 17). We have found no relation between oedema and either chloride or sodium levels in serum. The potassium deficiency may be very much increased by diarrhoea, as Darrow[17] first showed. In one case we found that at the height of the diarrhoea 85 per cent of the ingested potassium was being lost in the stools.

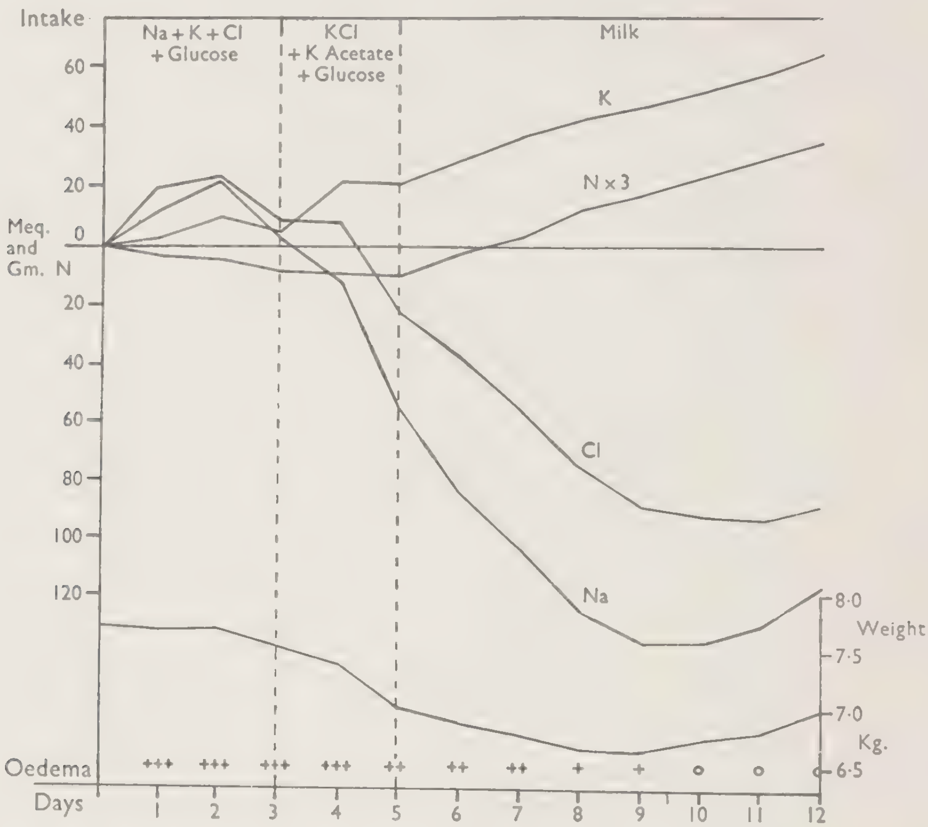

FIG. 17. Cumulative nitrogen and electrolyte balances during the treatment of kwashiorkor (A.B., 8 months old, 7·0 kg.). The figure shows that there may be retention of potassium with a negative nitrogen balance (days 3–5).

SÉNÉCAL: How much potassium do you give?

HANSEN: 4 g. of potassium chloride a day.

FREMONT-SMITH: Are you suggesting that part of the benefit of protein feeding is the potassium that goes with it, and that gets rid of the oedema?

HANSEN: Yes. I think that the electrolytes are as important as the nitrogen.

GYORGY: But can you separate potassium and protein? If I am not mistaken, any negative nitrogen balance will lead to negative potassium balance. That is, I think, a kind of hen-and-egg proposition.

HANSEN: I think they can be separated. Figures 16 and 17 show a retention of potassium without a parallel retention of nitrogen.

GYORGY: Only for 1 week. During the development of kwashiorkor, there is an absolutely essential sequence of continuous negative nitrogen balance.

HANSEN: Yes, and with that you get an even greater negative potassium balance.

WATERLOW: The ratio of potassium to nitrogen retention in your cases during treatment seems to be different from the ratio during normal growth.

HANSEN: Yes. In the first 4 days of treatment the ratio of potassium to nitrogen retention is about 6 meq. per g. nitrogen. After that the ratio falls to 3 meq. potassium per g. nitrogen, which is approximately the ratio in muscle.

PLATT: From the point of view of dietary requirements, it is worth noting that in the diets of under-developed countries it is usual to get relatively high intakes of potassium, particularly when so-called native salts—literally potashes—are used instead of common salt. Therefore I think Dr. Gyorgy is right, that this is an accompaniment of the protein insufficiency and not primarily a potassium deficiency.

HANSEN: I accept that. I don't think it is a primary dietary deficiency, but a secondary deficiency caused by diarrhoea.

Blood Urea

GOPALAN: Dr. Sénécal, did you measure the blood urea? Did you find the changes that Dr. Dean has described? [8, 18]

SÉNÉCAL: Yes. On admission the blood urea in my cases is low, averaging 16 mg. per 100 ml. (Table 30). We have on occasion found levels as low as 10 mg. On treatment it rises to an average of 30 mg. per 100 ml. and in some cases much higher, to about 80 mg. If that happens we reduce the protein intake, and in a few days the urea falls to a normal level.

Table 30 also shows the changes in total serum protein. The rapid response to treatment suggests that the serum protein level might be used to indicate whether the dietary protein intake is adequate. To be precise, we think it is not the total protein that is important, but rather the changes in electrophoretic pattern (a rise in α_1 and α_2 and a fall in β globulin).

TABLE 30

Concentration of total protein and urea in serum of infants with kwashiorkor

Period of treatment		Total protein (g. per 100 ml.)	Urea (mg. per 100 ml.)
On admission:	mean	4·28	16
	range	2·6–6·2	10–32
5th–12th day:	mean	6·02	30
	range	3·2–7·7	16–60
After 1 month:	mean	7·4	31
	range	6·1–8·6	10–48

PLATT: May I suggest that these high blood ureas are the result of damage to the tissues by giving too much protein?

DEAN: No.

PLATT: Well, I may suggest it. I may be wrong.

FREMONT-SMITH: You may, indeed.

PLATT: If you put rats on a low-protein, high-carbohydrate diet, and then after a time give relatively large doses of protein, you constantly and almost to the day produce damage to the kidney.

FREMONT-SMITH: Then this is a translation of experiments made in the rat to what we see in the child. But we know that a great many things occurring in one animal do not occur in another.

PLATT: Quite so; but is there any other explanation for these abnormally high blood ureas?

DEAN: I don't myself think they are important. I have never been able to detect any sign of kidney damage in the children who come to us.

PLATT: The deprivation phase does not damage. It is when you give protein to the deprived animal that you get the damage.

MAYER: But Dr. Sénécal was only giving 25 per cent of his calories as protein, and this surely would not damage any animal.

Cycles of Good and Bad Feeding

PLATT: There is another aspect to this sequence of low levels followed by relatively high levels, which should be realized by people who don't know the problem in the field. A phenomenon common in Africa is the "hungry season" —a period of hunger followed by a season of comparative plenty. This seasonal variation in the supply of food has a direct effect on the growth curve of children throughout the year (Figure 18).

The rather severe experiments I have mentioned were done in order to study the effects of this cycle. You might get sequences within the year of two sorts of damage—damage due to shortage, and then damage caused by a failure of adaptation, when the organism gets relatively more of something—maybe not enough, but still more than the state of his tissues at the time can tolerate.

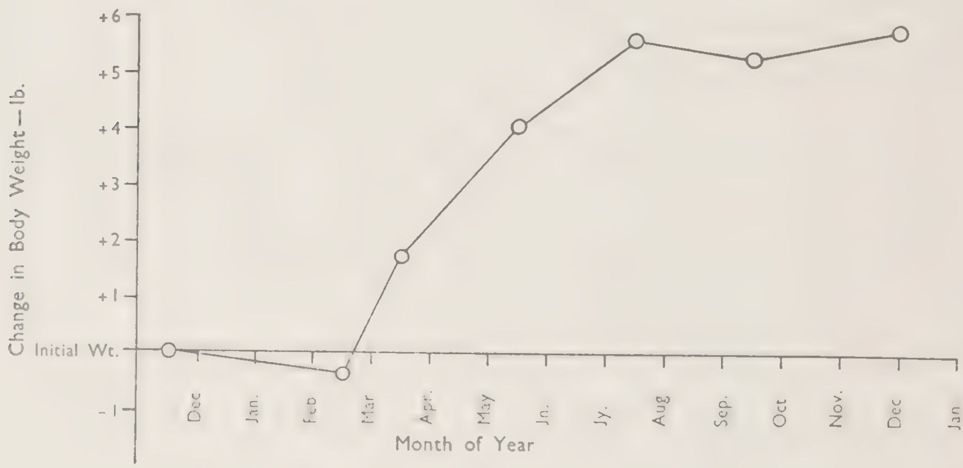

FIG. 18. Typical annual growth curve of African village children. (Gain or loss in weight shown throughout the year for 25 boys and 27 girls.)

DEAN: This is entirely hypothetical.

AYKROYD: I think that is a bit far-fetched.

HOLT: I would like to support the idea that a sudden increase of protein is bad, at least in the rat. I haven't made any histological examinations, but it can be followed by death of the animal. If allowed access to protein alone the malnourished rat will refuse it almost completely at first, and will step up his intake very gradually as he adapts to such a diet.

WATERLOW: I think Dr. Platt's point is important, and that he is right to sound this note of warning. I have seen slides in his laboratory from animals which had gone through this cycle of low and high feeding, and the effect on the pancreas and liver was very bad—much worse than the effect of low feeding by itself.

DEAN: Have you seen any children suffer during the good season because they are getting more food than they had during the hungry season?

SCRIMSHAW: Gomez[19] has described what he calls the "recovery syndrome", in which there is a temporary increase in the size of the liver, but he regards it as completely benign.

DEAN: Only Gomez and a few people in Central America have seen this recovery syndrome, although it has been looked for very carefully. It appears to be rather a special phenomenon.

HOLT: I have seen it in New York and I think it is distinctly not benign. You can show impairment of liver function while it is developing.

GYORGY: It has been repeatedly stated that kwashiorkor is based on lack of protein and that in consequence we should treat it with an *excess* of protein. The same fallacious logic has been used in liver disease, with the result that a high protein diet has been found detrimental in patients on the verge of hepatic decompensation.[20, 21] Best and his colleagues[22] have presented evidence that a sudden increase in protein in the diet of rats previously on a low protein diet will increase the fat in the liver tremendously.

SÉNÉCAL: But in the child with kwashiorkor there is an extremely rapid disappearance of fat from the liver. Moreover, I think that in the condition described by Gomez the liver was not fatty.

ELVEHJEM: What is the rationale of high protein feeding? Why give protein at many times the normal level? It reminds me of our early experience with vitamins; vitamin therapy was given in 10, 20 or 100 times the required dosage, and we seem to be going through the same cycle here. The results are interesting, but why don't you base your experiments on past experience?

DEAN: These high figures that I have mentioned were part of an experimental programme to find out how much protein was necessary in the treatment of kwashiorkor. I don't think that enormous quantities of protein are in fact needed.

SÉNÉCAL: I believe, however, that a high level of protein intake shortens the time of treatment and helps to avoid relapses.

GOPALAN: I agree that it is important to decide the optimum amount of protein to give these children. We discussed this question in Jamaica, and I quoted the evidence from India which suggests that there is no room for exceptionally high protein intakes, transfusions, etc., in the treatment of kwashiorkor. I regard clinical management as a tool to help us understand the public health problem—the control and prevention of the disease in the field. That is what matters for the purpose of this conference.

REQUIREMENTS OF INFANTS IN TERMS OF MILK PROTEIN

TERROINE: I may be excused, perhaps, if as a Frenchman I seek to introduce some order into the discussion. Our principal aim is to reach agreement on guiding principles that would enable the international agencies to make useful and practical recommendations applicable in all countries. The discussion as it is proceeding now would certainly not help them in that task. Let us come back

to the problem as it was outlined by Dr. Aykroyd and try to reach agreement on principles, methods and a basis of evaluation.

Rubner said a long time ago that there are as many figures for protein requirements as, in fact, there are different proteins. It has been mentioned that you can have kwashiorkor with an intake of 1 g. of protein per kg., and you can have it with an intake of 2 g. Why? Because in all these cases we are not dealing with the same protein. It is clear, in fact, that we cannot have just one figure for protein requirements.

What are we to do, then? I think it might help if we were to select one protein food as a basis and try to fix a definite figure for requirements in terms of that basic protein food. What should we choose as the basic protein? The best protein, of course, is white of egg, but perhaps it would be more practical to take milk as our basic protein food. If we could agree on a definite figure for the requirements of babies, children, pregnant women and all other specific cases in terms of milk—and I mean cow's milk—we could then work out afterwards a conversion coefficient and apply it in all the different regions in relation to the main sources of protein used there.

King: I think that is a very excellent suggestion, and as we have begun by considering the requirements of infants, it is all the more suitable that we should think in terms of milk as the basic protein.

Gyorgy: I should like to correct a misunderstanding that may have arisen from the first day's discussion. Our own studies dealt mainly with nitrogen balance, but I did not mean to imply that nitrogen balance alone is what we have to have in children. We have to have growth and, as we discussed yesterday, there may be other factors too, such as resistance to disease.

If we do not speak of minimal or optimal, but only of the *normal* protein requirement, then with human milk—and cow's milk is very close to it—1·6–1·9 g. of protein per kg. is the requirement for protein in an infant. The National Research Council's Food and Nutrition Board[23] recommends, at least for the artificially fed infant, 3·5 g. of protein per kg. per day. I take great exception to that because there are no facts to prove it.

King: The Food and Nutrition Board of the National Research Council at their last meeting agreed that the present value in the published table is probably too high, and that it was the result of inadequate and misinterpreted information. Upon finding that the American Academy of Pediatrics has a group and a committee working actively on this problem, they deferred making an artificia change in the recommended dietary allowances until the Academy can gathe the information, and complete research in progress.

Gyorgy: For our own particular purpose here, it is extremely important t make a very clear, exact, scientific statement that with 2 g.—and Dr. Holt agree on 2 g.—

Holt: Yes, with the slight qualification that a little more may be needed i the neonatal period.

Gyorgy: —with 2 g. per kg. per day (human milk or cow's milk), even growing infant, during the first 6 months of life, has the requirement it need We may assume *a priori* that with the reduced growth rate after the first 6 or months, the requirement will be less—that is, in terms of proteins of hig biological value like cow's milk or human milk. Following Professor Terroine' suggestion, I purposely omit the discussion of plant protein. Therefore, for cow' milk protein, say, 2 g. per kg. would be ample for the first 4 years of life an probably even less would be enough for older children.

I make this statement because, for the purposes of the agencies interested in this problem, it is important that we be modest in our goals, provided those goals are still based on exact scientific foundations.

DEAN: I am still disturbed that you equate cow's milk and human milk. You seem to have assumed a great responsibility.

GYORGY: The responsibility is already assumed in some sense, because we have a large number of data on infants fed on cow's milk, with a protein intake as low as 2 or 2·5 g. per kg.—and I think Dr. Holt would again concur with me, that this is enough.

KING: How close are we to getting agreement on the requirements for infants? Dr. Autret, would you recapitulate from the data that have been presented what you would suggest as an adequate protein intake?

AUTRET: We might, for instance, calculate from Holemans' data (Figure 12, p. 78) the quantity of protein the children are receiving just at the time when the growth curve is going up at 12 months, when presumably the protein consumption meets or even surpasses normal requirements. At 18 months, when the growth curve flattens, the quantity of protein from the breast, from skimmed milk and from family diet, is apparently unsatisfactory. We might apply the same approach for the child at 6 months, at 2 years and at 3 years. On this basis the requirement at 15 months would be about 1·5 g. of protein per kg. body weight. There is, of course, some uncertainty about this estimate, because we don't know how much the child might have picked up from the family diet.

KING: Is there general agreement on this figure?

ELVEHJEM: I think that 1·5 g. per kg. sounds reasonable on the basis of the evidence that has been presented.

DE MAEYER: Between 12 and 24 months, 1·5 g. per kg. would in my opinion be enough if we are thinking in terms of milk protein.

PLATT: I wouldn't accept a minimum figure. I think there is one figure, and one figure only, neither minimal nor optimal, but adequate. I accept the data in Dr. Holt's Table 5, columns 2 and 3 (i.e. 1·6–1·8 g. per kg.), as an objective for the first 4 to 6 months of the infant's life.

HOLT: I think it is unfortunate to have one figure stated for the requirement of infants at all ages, because the requirement is greater in the first month or two than later. If you make an analysis of breast milk during the first 2 months and on into the third month, the early breast milk has a higher protein concentration. This is the so-called transitional milk. We used to think that was just a carry-over from colostrum, but I think it probably has some real function, and that the child's protein requirements are higher at that time.

In my opinion therefore, for the first 2 months or so of life the requirement would be 2·2–2·5 g. per kg., and then, perhaps, 2 g., and, toward the end of the first year, I think 1·5 g.

MAYER: Assuming that this is all milk or mostly milk protein?

HOLT: I am assuming milk protein, and I am assuming no great difference between cow's milk and breast milk. I think that the differences are minimal.

GYORGY: I agree with Dr. Holt's statement. I would accept 2·2 g. per kg. for the first month as an optimal requirement; then 2 g. for the months 2 to 5, and 1·5 g. for later, provided this is expressed—as very clearly and succinctly stated by Professor Terroine—as milk protein. I agree with my paediatric colleague, Dr. Holt, that the difference in protein utilization between human milk and cow's milk is not too significant.

MAYER: I think that is much more acceptable than the usual figure of 4 g per kg.

DARBY: As we move away from milk to essentially vegetable diets, we shal have to increase our estimate of requirements. There may not be a thoroughl defensible scientific basis for doing this, but it must be done for the sake o safety.

TERROINE: I would like to emphasize again that we must adopt milk as ou basic standard of reference. Only from that can we calculate the various correc tion coefficients to be used for different protein foods.

DEAN: I want to take up the astonishing statement that cow's milk is com parable to human milk. This is a matter of great practical importance, becaus we are in danger of saying that we can get something which is as good as th natural food for infants—breast milk. I agree with the figure for the requiremen of protein from breast milk, because that is founded on intakes. I am not sur that our figures for cow's milk are so firmly based.

TERROINE: The comparison between human milk and cow's milk is a extremely complicated problem. The composition of the two is admittedl entirely different. They differ in their content of protein, of lactose and of fats As regards the protein, cow's milk is very rich in casein and poor in lactalbumin whereas human milk is rich in lactalbumin. But if we consider the biologica value of whole milk as a source of protein, we find only minor differences. There fore if we adopt milk as our standard of reference, there is no great objectio to using cow's milk rather than human milk.*

WATERLOW: It is striking that there is a close correspondence between th minimum requirement given by Dr. Holt in his Table 5 (p. 22), the optimum requirement for normal growth proposed by Dr. Gyorgy, and the figure pu forward by Dr. Autret, which was based on observed intakes. This last figure however, clearly did not leave much margin.

DEAN: Yes, it is interesting to see this convergence of opinion between th people doing experimental work which is founded upon minimum requirements and the people who take intakes as a standard of reference. It seems to me tha the acceptance of intakes in this limited way offers us a practical standard, no only for this age but also for other ages.

HOLT: However, Dr. Autret's and Dr. De Maeyer's growth curves (Figure 12 and 13) were not quite normal. They were subnormal.

AUTRET: Yes, but they show a tremendous improvement compared with th children who got no supplementary food.

GOPALAN: In all the discussion so far, the adequacy of a protein intake ha been judged from the growth observed on that intake. But in doing this one is ap to run into several fallacies. If a group of children has a low protein intak and also a low growth-rate, we can't say that the one necessarily follows fron the other. A low protein intake is invariably associated with a diminution i one or more of the other constituents of the diet, and with bad environmenta conditions. Therefore I think this question can only be answered by controlle studies in which children are put on different levels of protein with diets an in environments that are otherwise the same.

GYORGY: Yes, that must be part of the action programme, but in the mean time we can learn much from these field studies.

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* The biological value of milk as a source of protein—its protein efficiency, *sensu stricto*—must not be confused with all its other properties, such as digestibility, vitamin and miner content, etc., which may differ considerably from one species to another. (Terroine.)

Amount of Protein from Breast Milk

SENECAL: Our own findings agree with the figures suggested by Dr. Holt: that is, 2·2 g. per kg. for the first 2 months of life; after that 2 g., and then a diminishing amount, approaching 1·5 g. at the end of 1 year. Since in Dakar we find that the growth curve during the first 6 months is even better than in America or Europe, it would seem that that amount is quite satisfactory.

I have perhaps an explanation for the fall in the growth curve at 5 or 6 months. According to our measurements, at the beginning of the lactation period the protein content of the milk is 1·7 g. per 100 ml., it falls at 6 months to about 1·2 or 1·1 g. per 100 ml., and then tends to rise progressively until at 18 months it reaches 1·6 g. per 100 ml. There may well be a relation between the growth curve of the infant and the protein content of the milk, but we have not enough figures to prove this statistically.

VERHOESTRAETE: When I asked Dr. De Maeyer what was the average protein content of the milk from his mothers, he said about 1 g. per 100 ml. Dr. Scrimshaw gave an average figure of 1·2 g. per 100 ml. Now I think that the observations that have been made on breast-feeding in Africa and Central America show an average milk output of 500–600 ml. per day. This means that a baby of 4–5 months, weighing say 6 kg., will be getting about 7 g. of protein which is considerably less than the 2 g. per kg. per day that has been proposed as the requirement. Yet, as we have seen, babies in Senegal, in the Kivu district of the Congo, and in Guatemala grow normally up to about 6 months. This suggests, then, that adequate growth may occur with intakes less than 1·5 g. per kg.

HOLT: I don't believe that the child who gets only 500 ml. of breast milk and no other supplement will grow normally and double its birth weight at 4½ months. 500 ml. is too little. According to our observations, healthy well-nourished mothers secrete the following amounts of breast milk:

Age of child	*Amount of milk*
1 week	300–400 ml.
2 ,,	400–500 ,,
3 ,,	450–700 ,,
4 ,,	500–800 ,,
4 months	600–1,000 ,,
6 ,,	700–1,150 ,,

I think that 1·5 g. per kg. may be too low for a child of 6 months, though it may be enough at 1 year.

KING: May I summarize the outcome of the discussion on the requirements of infants? It is satisfactory to find that our two paediatricians are in agreement. Dr. Holt has put forward the following figures for the protein requirement of the infant in terms of human milk: 2·2 g. per kg. at birth, falling gradually to 2·0 g. per kg. at 2 months, and then to 1·5 g. per kg. at 1 year.

There is no reason to suppose that the requirements are substantially different if based on cow's milk instead of human milk.

There is some evidence from Africa and Central America that normal growth may occur for the first 5 or 6 months of life on intakes of less than 1·5 g. per kg. This, however, needs confirmation. More measurements are needed of the actual protein intake of these infants, both from breast milk and from other supplementary sources.

Are Dr. Holt's figures accepted? (General agreement was expressed.)

Protein Requirement and Calorie Intake

CATRON: You might, however, consider expressing the protein requirement on the basis of caloric intake. In chick nutrition, for instance, the protein energy ratio is very important.

GOPALAN: That is a valuable point. While I accept in principle the figures that have been put forward, as applying to a normal child, in under-developed countries they may not be applicable because they are based on body weight. Is it right to use that as a yardstick, since growth itself is determined by the adequacy of the protein intake? In the under-developed countries I think we should calculate a child's protein requirements not on the basis of its actual body weight, which is probably too low, but on the expected normal weight of a child of the same age in that particular community.

KING: Genetic factors must also be taken into account.

HANSEN: I think it would be dangerous to give food at the level of the expected weight to a child who was half that weight. You would get digestive troubles immediately.

GOPALAN: I concede that we may have to work up to the ideal slowly.

MAYER: This same question arose in connection with calorie requirements. The international committee decided that as a matter of principle the requirements should be based on age groups rather than on weight.[24] It is obviously impossible, or it would be much more difficult, to stuff the theoretical calorie requirement into a child who is very much underweight and perhaps less active than normal. But I don't think that the same consideration necessarily applies to protein. In fact, I thought one reason why we are so much concerned with protein supplements and concentrates is that we might be able to enrich a diet, specifically with protein, without having to increase the calories to an uncomfortable level.

SCRIMSHAW: But is there any evidence that the child would be able to use all this extra protein?

MAYER: There is no evidence that it would be immediately possible, but one would hope that the intake could gradually be built up to the ideal level.

KING: This discussion has helped to define a number of gaps in our knowledge, but at least we have been able to reach a measure of agreement on the requirements of infants. However, there have been two factors making things easy for us. First, we have a ready-made standard in the form of growth, by which to judge whether the requirements are being met. Admittedly, as Dr. Gopalan has pointed out, this standard has its pitfalls, but at least it is a practical guide. Secondly, our reference protein, milk, is the protein actually consumed. As we move on to older children and adults, both these things cease to be true. From one year, or even earlier, the child gets a proportion of its protein from sources other than milk, and adults in most parts of the world get no milk at all. In adults obviously growth cannot be used as the criterion of requirement, and we have to consider the validity of other criteria, such as nitrogen balance.

SPECIAL REQUIREMENTS OF CHILDREN AGED 1 TO 5 YEARS

KING: I suggest that we now go on to discuss the requirements of children aged 1–5 years, and then of older children, adults, and pregnant and lactating women, bearing in mind these matters of principle that I have touched on.

SCRIMSHAW: At the same time, we must bear in mind that this is an ideal conception. You might not get any better results if you give all animal protein than if part of the requirement were covered by vegetable protein.

KING: Assuming good-quality protein, and waiving where it comes from, how much protein should such a child have?

DEAN: Miss Widdowson[25] collected some figures for the diets of children before the war in England, which include the proportions of animal and vegetable proteins. May I quote those, as a starting point for this discussion?

The children were from 18 to 30 months old. At 18 months they got on the average 40 g. of protein a day, of which 70 per cent was from animal sources and 47 per cent—i.e. nearly half—was from milk. At the age of 30 months the children were getting 45 g. of total protein, of which 65 per cent was from animal sources and 34 per cent, or one third, from milk. These were healthy children who were growing well. I know the theoretical objections to equating intakes with requirements, but I would say that these figures give us a reasonable target to aim at, and I would suggest that discussion centre on them.

SCRIMSHAW: What were the children's weights?

DEAN: At 18 months the average weight was 12 kg., which gives an intake of 3·3 g. per kg. At 30 months the weight was 15 kg., giving an intake of 3·0 g. per kg.

GYORGY: I cannot see why, because they got 3 g. per kg., that should be their protein requirement when we agreed that at the end of the first year the requirement is 1·5 g.

DEAN: I am quite prepared to defend that, from my own experience. In the trials I made in Germany[26] I arrived at something like the same figures for total protein intakes in children growing well, who were being fed adequately as far as I could tell.

AYKROYD: The fact that British children grew well on such a diet—how does that help us in Central America and India and Africa?

DEAN: You are trying to define the amount of protein needed. I have told you the amount of protein on which some children grow well. Doesn't that help?

FREMONT-SMITH: I think it is very important, if we don't have other data, to know that a certain intake will produce satisfactory growth; then we can work down from that.

MAYER: Dr. Davis and I have compiled a table of published results from various sources[9] (Appendix I). Most of them are from studies of the type cited by Dr. Dean—that is, intakes on which children were found to grow well—and the requirements are of the same order of magnitude. There are a few studies in which children have been found to grow well on lower intakes.

There is another approach which we have not tried in detail, but which confirms the idea that requirements are in fact lower than those given in the table as "optimal". In the Maternal and Child Health Department of the Harvard School of Public Health, Dr. Stuart and Mrs. Burke have examined a large number of children from birth to 20 months old and have obtained very complete dietary data as well as measurements of growth. We have found amongst them children who had adequate caloric intakes, but lower protein intakes than the average.

DEAN: Dr. Widdowson found in each age group variation in intake between individuals of the order of 100 per cent; in the 2–3 year age group, for example, the caloric intake ranged from 959 to 1,929 calories per day, and the protein intake from 29 to 57 g.[25]

MAYER: Yes, but what we were after was children who didn't have lower caloric intakes, but had lower protein intakes, and yet were growing normally.

I believe from this one can get a valid measure of protein requirements. This meets the point made earlier by Dr. Gopalan.

DARBY: The Food and Nutrition Board, in their last revision of recommended allowances, made this statement[23]: "The recommendations for children after infancy are based upon balance studies, as found in the literature. The data indicate that the protein need per kilogram decreases from 2·5–3 g. per kg. in early childhood to 1·5–2 g. per kg. in late childhood and adolescence, and to approximately 1 g. per kg. for adults; the values in the tables approximate these amounts." (See Appendix II.)

The 1950 Canadian standards[27] also recommend 2·5–3 g. of protein per kg. for the age group 2–4 years. The basis is stated to be "survey data in which normal growth was the criterion of adequacy". I think there are very few other data on this age group. As I recall it, we had to confine our reasoning to a few balance studies which were available, plus a good deal of the kind of data cited by Dr. Dean and summarized by Dr. Mayer.* All the recommendations are around 2–3 g., or a little above 3 g. per kg. I doubt, therefore, that we are in a position to make as good an approximation of the protein requirement for this age-group as we were for the breast-fed infant.

FREMONT-SMITH: May I raise a question along another line? What is the highest figure that we know to be *in*adequate? This gives another approach to the problem.

SCRIMSHAW: In the group of children presented in Table 22 (p. 76), the average daily intake between 1 and 2 years of age was 15 g. of protein, almost entirely of vegetable origin, and almost entirely from corn. Their growth was very poor and their weight very low for their age. However, I admit that these data are inadequate, because they are based on intake studies carried out for 2 days only.

WATERLOW: At the Jamaica Conference[8] (p. 81, Figure 10) Dr. Rhodes showed a diagram of the intakes of five healthy control children of normal height and weight for their age; these were pre-school children about 3 or 4 years old, and their highest protein intake was roughly 65 per cent of the NRC standard.

GOPALAN: We also have some data on apparently healthy children. Between 1 and 2 years of age the protein intake was about 25 to 30 g. a day, and from 2 to 3 years it was 30 to 35 g. The protein was derived mainly from cereals and to a certain extent from pulses. These children seemed to be growing much better than children on 12·5 or 15 g. of protein, yet their growth curves were generally lower than those of European or American children. The point has to be remembered that this difference may not be related to the quantity of protein but to the quality.

AUTRET: Information of the same kind is available from several parts of the world,† but the results seem to be somewhat contradictory. In New Caledonia, for instance, Malcolm[31] found that at 24 months children were approximately

* See, for example, the observations of Virginia A. Beal[28] in a study of the usual dietary consumption of a group of healthy children in the United States. She reports a consistent decrease in estimated protein intake per kg. of body weight through the fifth year. The median protein intake from 1 year through 3 years of age decreased from some 4 g. per kg. to approximately 3 g. per kg. Icie G. Macy[29] from accurately determined intakes of children found good nitrogen retention by 4-year-olds on intakes of 3·5 g. per kg. It is to be emphasized that such studies do not give minimal estimates of good intakes. They merely indicate that a given level is conducive to normal growth and N retention. (W. J. Darby.)

† Baptist and de Mel[30] studied the heights and weights of 23 Ceylonese children aged 1–6 years, after they had been given an entirely vegetable diet ('test'), or the same diet supplemented with skimmed milk ('control') for 17 weeks. The mean intake of total protein in the age-group 2½–6 years was 2·4–1·6 (test) and 3·0–2·1 (control) g. per kg. per day. There was no statistically significant difference between the two groups as a whole. (Editor.)

the same weight and height as Australian children; yet a quantitative study (on 4 children only) showed a calorie intake of only one half to two thirds the recommended allowance, and a protein intake of only 10–19 g. a day, mostly of vegetable origin. In Peru, Huenemann and Collazos[32, 33] found that all children over the age of 10 months were small, and nearly all showed signs of nutritional deficiency: here the mean calorie intake was 90 per cent, and the protein intake 62 per cent, of the NRC allowances.

PLATT: I think that the few protein intake figures available for African children on the whole relate rather well with the observations on growth. The heights and weights of Nyasaland village infants and young children up to 5 years are almost the same as those of Glasgow slum children in 1919–23. In one of these villages—a hill village—the children from 2 to 10 years got from 2·5 to 3 g. protein per kg. body weight.

AYKROYD: I want to repeat the point I made yesterday: if you take a figure of 3 g. per kg. for the requirement of young children, it is outside practical possibilities in most of the under-developed countries.

CRUICKSHANK: Could we, perhaps, start from the other end? We have agreed that 1·5 g. of protein per kg. at the age of 1 year is adequate, and this is a period of very rapid growth. Why should the need be increased after 1 year? What are the additional metabolic demands on the child?

AYKROYD: There is surely no reason to suppose a large jump in requirements between the ages of 1 and 6, rather than a smooth transition.

ELVEHJEM: It is a matter of food intake. As the caloric requirement goes up, one way of getting the calories is to supply food rich in protein. The figures quoted by Dr. Dean and Dr. Darby don't surprise me, but they are probably far above the minimum requirement of protein. These are typical intakes in countries where for a couple of decades we have been preaching against sugar and other carbohydrates mainly in order to protect the teeth.

STARE: When children are more than a year old, in practice we try to feed them as though they were adults, and then we meet this problem: if the child at 1 to 3 years of age or the adult at 50 years of age is getting enough calories to show a reasonable rate of growth or to maintain an adult body weight—enough calories from foods other than more or less refined carbohydrates—then protein is automatically taken care of in good quality, and probably in a quantity that is far above the minimum needs.

DEAN: I think that this change in dietary habits is likely to be a reflection of economic levels, because animal protein is always expensive.

CATRON: We find in pig-feeding that pigs will eat more protein than they actually need for optimum growth, and certainly more protein than is needed for minimum growth, if they are offered free choice.

ELVEHJEM: In the first session I described some experiments on rats which indicate that fat does have a considerable sparing effect on protein; so this might be an explanation for a sudden increase in protein requirement as you go from a milk diet with a relatively high fat content to the type of diet Dr. Dean was talking about.

CATRON: I think we should try to get back to protein requirements on a caloric basis, especially when considering the calorie intake in different countries, such as in India versus Germany or here. It is a relationship which we see developing in the animal field.

GOPALAN: The point has already come up that in undernourished communities, where the body weight of the children tends to be low, it may be fallacious to

calculate the requirement on the basis of body weight. After all, the body weight is itself determined to some extent by the level of protein intake.

MAYER: The question whether requirements should be expressed as g. per kg., or g. per 100 g. of diet, etc., is not just a matter of choice of expression: it is a matter of purpose, of what you want to do with your data.

If you are evaluating a diet, then I think the proper way to express it is in percentage of calories represented by protein, because that tells you whether it is a good diet or a bad diet from the point of view of protein nutrition. If, on the other hand, you are interested in supplementary feeding programmes, then you have a child of a certain age or a certain weight, and you want to increase his intake almost solely with protein. In that case, I think that g. per kg. or g. per 100 g. of food is the proper approach. But I don't think it is just a philosophical question as to which is better. It is a very pragmatic question, and depends on what you want to do.

FREMONT-SMITH: I want to ask another question; is there any possibility that the increased activity of the child as it comes to the age of walking and running would cause any greater demand for protein? I know the general assumption is that the need is all for calories, but I just want to raise the question.

MAYER: I think that the bulk of experimental evidence over the past hundred years is that nobody has ever been able to demonstrate an increased requirement of protein as the result of exercise. You have an increased requirement for calories, which in practice means an increased *intake* of protein, but I don't think there is an increased *requirement*.

TERROINE: I think that all physiologists agree, and have agreed for a very long time, that even a very large increase in a subject's activity and expenditure of energy has only a very slight effect on his nitrogen expenditure.

FREMONT-SMITH: But I am gently challenging this. I know that all physiologists agree on this, but what I am asking is, are there any data that show it to be true for *growing children* as opposed to adults? Are there any data on growing animals that show it to be true for growing animals? I am assuming that the caloric intake is satisfactory.

ELVEHJEM: There is plenty of evidence for animals.

MAYER: There is good evidence that for a given caloric intake, there is better nitrogen retention if the animal is exercised; so, if anything, exercise would decrease the requirement.

TERROINE: I would like to remind you that the first demonstration that even very heavy work has a negligible effect on nitrogen expenditure dates, if I am not mistaken, from 1878. This is the classical observation of Fick and Wislicenius. Again, as far back as 1910, Thomas[34] showed, in a series of experiments carried out with the utmost rigour, that in man the endogenous nitrogen expenditure increased from 2·72 g. at rest to 2·78 g. at a work rate of 120,000 kg. a day—in other words, a negligible difference.

FREMONT-SMITH: But in children?

TERROINE: There is no reason to suppose that it would be any different in children. Undoubtedly nitrogen expenditure would be proportionately greater in children than in adults. It obeys the law formulated by me in 1927 and soon afterwards confirmed by Smuts and by Brody in the United States. According to this law, nitrogen expenditure per unit body weight is strictly proportional to the basal metabolic rate. Any activity superimposed on the BMR will only have a very small effect on nitrogen loss.

KING: I think it is time to try and summarize this discussion. We still do not have any basic data which give us the requirement in the 1–5 age group. The records of dietary intakes in normally growing healthy children lead to a figure of about 3 g. per kg. Yet we agreed that at 1 year 1·5 g. per kg. should be enough. The children quoted by Dr. Dean were getting 1·5 g. per kg. specifically from milk, and on top of that an equal amount of protein from other sources, some of it animal. As Dr. Cruickshank pointed out, this does not seem at all logical.

CRUICKSHANK: If we can't, as it seems we can't, get adequate data for the pre-school child, can we not consider the school child, for whom we have good data, and try to extrapolate back from there? In this way we might get at least a working figure.

PLATT: I think it is safer if we think of interpolating between 1 year and 5, rather than extrapolating backwards from 5.

HOLT: I have heard no reason brought forward why the intake of protein on a body weight basis should increase from the age of 1 to the age of 5 years. The activity of children increases and they need more calories, but, as has been pointed out by Dr. Mayer, they don't need more protein per unit body weight. Couldn't we accept the figure of 1·5 g. per kg. tentatively as the basic requirement on a milk diet? This will be a standard of reference for the requirements on mixed diets containing various proportions of less valuable proteins. We are trying to define the minimum; I think 1·5 g. is an excellent tentative basis for us to start from, which we can fill in by working backwards from the child of 5 years and over.

DEAN: It seems to me not to be a good basis because I, like yourself, Dr. Holt, have actually fed children and know what they take. I admit I have not tried to define the "minimum", but have simply given diets on which I know a child will grow well. I have merely, as you must have done, offered the child what it would conveniently take, and those figures that I have quoted from Dr. Widdowson fit in with my own experience.

HOLT: But we have been trying to define, as I understand it, minimal limits on a pure milk diet. That is the question.

DEAN: That is an entirely theoretical thing.

MAYER: I think Dr. Dean is perturbed by the lack of reality of discussing intakes solely in terms of milk protein, as a purely theoretical conception. I think we know enough about the supplementary value of proteins to assume that a diet containing a substantial proportion of animal protein, say, 50 per cent or more, probably has a biological value of the same order as a diet containing purely milk protein; therefore I think it is reasonable to base our standard on milk, and then to consider diets containing exclusively vegetable proteins.

Quality of Different Proteins

KING: Are we agreed, then, that for this age group (1–5 years) an intake level in the range of 1–1·5 g. per kg. would be adequate on the basis of milk protein? Then we can go on to consider the factor of equality and the degree of equivalence among proteins from all sources. A commonly accepted standard has been that half the protein intake should be in the form of animal protein. Would that target be satisfactory, even though it cannot be reached in many parts of the world for a long time?

HOLT: Are there data from which coefficients could be obtained and applied to vegetable protein diets in common use?

TERROINE: I agree that we must proceed by means of comparisons. I have been struck by one fact; that is, that we seem to have much less information on the way to feed children than we have on cattle feeding. That might be explained, of course, by the fact that cattle are so much more profitable. We also have many data on the comparative value of various proteins fed to pigs, especially during their younger periods, when they grow at a much more rapid rate than human beings. We could, perhaps, use data that already exist on pigs to work out a reasonable coefficient relating other proteins to milk; this could then be applied to human beings.

MAYER: Since the development of the pig is very much more rapid than that of man, it tends to exaggerate differences between the biological values of proteins. Therefore, if one took a coefficient established for the pig, there would be a considerable margin of safety in applying it to the human child.

BENGOA: I agree with Professor Terroine's suggestion. Children can be divided into three categories as regards their food intake: first, those receiving at least 50 per cent of their protein from animal sources; secondly, a much poorer group, that receives less than 25 per cent of animal protein, perhaps down to none; and an intermediate group which gets between 25 and 50 per cent of animal protein. Practically all the child populations in the world would fall into one of these three categories.

On the basis of the discussion today, in the first group you would end up with 1·5 g. of protein per kg. of body weight. Dr. Dean suggests 4·5 g. per kg. for the group receiving predominantly vegetable protein, i.e. less than 25 per cent animal protein in the diet. By interpolation between these two groups one would get a figure of 3 g. of protein per kg. By some formula of this kind we might arrive at recommendations suitable for all different countries.

ELVEHJEM: I would seriously object to such high levels, because 4·5 g. per kg. of a protein which produces an amino-acid imbalance might be much worse than 3 g. of good protein; so I think we must consider the balance and imbalance of amino-acids. Therefore, why try to establish conversion factors for proteins as such? Why not make our comparisons in terms of amino-acids?

DEAN: I don't think that is right. The figure of 4·5 g. per kg. is what children ate when I gave them a diet which consisted almost entirely of plant protein.[26]

ELVEHJEM: If that plant protein were from corn, my guess is that they would do better if you gave them 3·5 rather than 4·5 g. per kg.

DEAN: It was a mixture of wheat and soya, and I consider that the intakes were probably set at that high level because of the imperfections of the mixture. In your experimental conditions you can talk about pure amino-acids, but when you try to feed children on plant protein diets, there are a large number of variable factors that have to come into consideration, some of which have little to do with amino-acids.

ELVEHJEM: I maintain that if you calculated the amino-acid composition of your diets, you would find they were balanced, and that is why you got good results with 4·5 g. per kg. On the other hand, if you took the same intake, but used only corn, you would get into very serious imbalance.

DEAN: Yes. My diets were quite well balanced as regards amino-acids.

SCRIMSHAW: There is another point that supports Dr. Elvehjem's approach, and argues against settling for a given quantity of inferior protein, such as 4·5 g. per kg. Relatively small alterations in the combinations of vegetable proteins used in under-developed areas have been shown to improve the quality and greatly to reduce the quantity required.

GOPALAN: I should like to endorse the point of view which Dr. Elvehjem has advanced. We have done some work on adult volunteers—and I am speaking of adults now—using a combination of vegetable proteins—75 per cent cereals and 25 per cent pulses. With this combination the level of nitrogen retention is as satisfactory as with animal protein. In cereals the limiting essential amino-acid is lysine, but the deficiency can be more than made good by giving pulses. So I agree that we should work on the basis of amino-acid content, and not persist in talking of animal proteins and vegetable proteins.

SCRIMSHAW: I am glad that Dr. Gopalan has gone so far as to say that he can get mixtures of vegetable protein which are equal to animal protein, because our experience has been similar. We have not yet tested these mixtures on humans, but we have tested them on chicks and rats, and to a limited extent on young pigs. There seems to be no doubt that with plant products locally available in Central America we can arrive at economical mixtures of this kind.

MAYER: We have shown[9] a very striking agreement in the amino-acid content of the various diets which have been reported as satisfactory.

PLATT: But even in a well-balanced mixture some amino-acids may be only just sufficient. We have published[35] an evaluation of the amino-acids which are supplied by three typical African diets at the 1,000 calorie level, compared with the requirements proposed by Albanese.[36] I shall have more to say about these diets later (see Figure 19, p. 115). We can apply these figures to children, because it is important to recognize that in Africa children are often put straight on to an adult diet after weaning. The point I want to make now is this: if the child weighing 10 kg. is fed sufficient of an adult diet to provide 100 calories per kg., i.e. 1,000 calories per day, he will receive only two fifths of the amino-acids an adult receives when taking the diet at the 2,500 calories level daily. If, as indicated by Holt (p. 25), the child's requirement for essential amino-acids per kg. body weight is approximately ten times that of the adult, then a 10 kg. child will need twice as much of each amino-acid as a 50 kg. man. If there are any essential amino-acids which are only "just sufficient" in the 2,500 calorie diet to meet the adult requirements, then the 10 kg. child on the diet at the 1,000 calorie level will receive only one fifth of his estimated needs of these amino-acids. This simple example does I think give an idea of the magnitude of the gap which there may be between a child's needs and the supply of amino-acids when he is weaned from the breast on to an adult diet.

KING: Well, there have been several expressions of opinion that the best common denominator for evaluating plant proteins in relation to our reference protein—milk—is an estimate of the "available" amino-acids that these proteins will furnish. Under this heading we have to consider the amino-acid content, the digestibility, the risk of gross imbalance and possible deterioration caused by storage and processing. These matters will come up for discussion later, but I would like to re-emphasize the point made by Dr. Scrimshaw and Dr. Gopalan, that in making the evaluation we must take the mixed proteins of the diet as a whole, and not think in terms of single proteins.

We might now go on to consider the requirements of older children, adolescents and adults.

Requirements of Schoolchildren

DARBY: I will start the discussion by quoting again from the recommendations of the Food and Nutrition Board.[28] "The protein need per kg. decreases from 2·5–3 g. in early childhood, to 1·5–2 g. in late childhood and adolescence,

to approximately 1 g. for adults." Thus for a boy 10–12 years old, weighing 35 kg., the protein allowance was set at 70 g. The Canadian figures[27] are almost identical (see Appendix II) and so are those of the League of Nations.[37]

GYORGY: But again this doesn't fit. We were down to 1·5 g. per kg., including a proportion of vegetable protein, and now you put it up to 2 g. again.

DARBY: The difference is that the NRC figures represent an allowance attainable under North American dietary habits: this is something quite different from a minimum.

GYORGY: Yes, but we are interested in a recommendation for under-developed countries.

PLATT: I have some figures for the protein intake of children of three age-groups in a Nyasaland village. Maize was the staple of this diet (Appendix III, diet 1). The daily protein intake, and the sources from which this protein was derived, are shown in Table 31. We cannot judge the nutritional status of these children from a single measurement of body weight, since typically in this area the weight curve showed a seasonal rise followed by a fall or a stationary period. The children were repeatedly weighed during the years 1940–43.

TABLE 31

The total dietary protein and the amounts obtained from various categories of foods in the different age and sex groups in a Nyasaland village

Age and sex	Total protein (g.)	Source of protein (g. per head per day)					
		Cereals	Roots and tubers	Pulses	Fruit and vegetables	Animals and insects	Native beer
Men	69	42·9	0·3	10·5	4·1	3·6	7·8
Women	53	33·5	0·5	9·4	3·4	3·5	2·8
Boys 17–20 yr. ..	76	48·7	0·9	12·3	6·3	3·4	4·0
Girls 17–20 yr. ..	71	46·1	0·4	12·7	8·8	1·9	1·4
Boys 11–16 yr. ..	39	25·2	0·6	7·5	3·0	1·9	0·3
Girls 11–16 yr. ..	62	39·0	0·5	10·5	4·8	6·7	0·9
Children 3–10 yr. ..	44	26·0	0·4	7·9	2·9	5·7	0·9

It was found that in general they had much the same physique as London schoolchildren; their development was approximately the same as that of London children of the same age in 1905–12. They were not so advanced as present-day London children; however, I reserve judgement on the question of the biggest being the best!

The girls of 11–16 years seemed to do comparatively well. I estimate that they got about 2 g. of protein per kg. body weight: about two thirds of this was from maize and secondary staple foods. The boys of the same age were left to scrounge food for themselves. It is difficult to be sure that the values for food eaten by them are not low because of incomplete recordings. However, their low intake of protein (1–1·5 g. per kg.) is consistent with the observations on growth.

SCRIMSHAW: I have some similar data for schoolchildren in El Salvador. These are summarized in Table 32.

KING: Did these children grow normally?

TABLE 32

Protein intake of schoolchildren in El Salvador

Age group	No. of children	Protein intake (g. per day)	Animal protein (per cent)	Protein intake (g. per kg. body wt.)
6–8	31	37	17	2·0
9–10	26	40	15	1·8

SCRIMSHAW: No. They grew very poorly during their pre-school years, better during their early school years, and at a rate similar to that of well-nourished children during their later school years.

The figures in the last column of the table are calculated from the actual body weights. If they were based on the weights of well-nourished children of the same ages, they would, of course, come out much lower. In both age groups the intake is about 60 per cent of the NRC allowance. It seems that even though more than 50 per cent of the protein was derived from corn, and only a very small proportion from animal sources, this level of intake was enough in the older children, but not enough at 6–8 years.

KING: That was a protein mixture of very poor quality, and in terms of the reference protein the intake must have been much less than 2 g. per kg., yet you say that at 10 years old it seemed to be adequate.

SCRIMSHAW: Yes. It is interesting that the older children did not grow better when given a lunch containing additional animal protein, or a comparable mixture of soya and other vegetable proteins.[38, 39]

PLATT: Did you find no response to the extra lunch even in the younger children whose growth was sub-optimal? You wouldn't expect to find any response in the older children, if their growth was normal.

SCRIMSHAW: We haven't yet tried it. Most of the children given the extra lunch were in the older age group, because at that time we didn't know about this flattening of the growth curve in the younger children.

KING: To summarize, would it be reasonable to keep the requirement *in terms of the reference protein*, at 1·5 g. per kg. throughout childhood, and taper it off beyond adolescence to about 1 g.? Adolescence may impose additional demands which we ought to consider.

ADDITIONAL REQUIREMENTS IN ADOLESCENCE

MAYER: Having talked to a great many people who had something to do with rationing plans in various parts of Europe during the war, I would like to call attention to the frequent statement that adolescents are a more vulnerable group than was generally realized beforehand. For instance, Trémolières in France[40] found, after having surveyed a large group of children in 1944 and again in 1949, that undernutrition—which, of course, in this case meant a deficit of both calories and protein—was easily reversible in young children; in fact, the same children who had been underweight and underheight in 1945 were above pre-war standards in 1948. On the other hand, this reversibility did not obtain for adolescents, who never caught up in height and weight and had a very high incidence of tuberculosis.

DARBY: The various official recommendations for older children and adolescents are as follows:

Food and Nutrition Board[23]:	2 g. per kg. at 10 years, falling to 1·5 g. per kg. at 16–20 years.
Canadian Council on Nutrition[27]:	1·7 g. per kg. at 10 years, falling to 1·5 g. per kg. at 14 years.
League of Nations[37]:	2·5 g. per kg. from 12–15 years
	2·0 ,, ,, ,, ,, 15–17 ,,
	1·5 ,, ,, ,, ,, 17–21 ,,

The Food and Nutrition Board's figures were based on both balance and intake studies; the Canadian figures were derived from survey data. I believe the values for this age group have a firmer basis than some of the figures we have discussed up to now.

The Food and Nutrition Board, in discussing their figures, stated: "these generalizations should not obscure the fact that protein requirements are relatively high for the periods of rapid growth and lower during periods of slow growth. The time of these growth phases varies among individuals."

HOLT: Professor Terroine in his memorandum[11] has given figures for the rate at which body protein is laid down at different ages throughout the growth period. The rate of gain falls from early infancy, reaching a minimum between 5 and 8 years, and then rises slowly; there is a sudden jump, almost doubling the rate, at the age of 14 to 16 years. I wonder if that couldn't be taken as a guide in formulating requirements?

TERROINE: It is true that these figures reveal a very striking fact, as you have pointed out. I took the figures for the body weights of children at different ages from tables published by the League of Nations; on the basis of the known composition of the body I then calculated the amounts of protein gained. The results show that there are great irregularities in growth, with periods of maximum requirement or maximum retention, and confirm the point made by Dr. Mayer, that there are periods in adolescence when the need for proteins is very high. That group is undoubtedly very vulnerable.

DEAN: But these figures are calculations, aren't they? They are not the results of analysis. So how do you know that they represent gains of protein and not of some other constituent?

TERROINE: The composition of the organism is constant; the proportion of protein only varies if there is a very great increase in fat stores. This seldom happens in infants or adolescents; therefore we are justified in calculating the gain in protein from the gain in body weight, at least to get an average figure.

DEAN: That is an assumption. Adolescence is the period of life when there are the greatest changes in endocrine balance; these might be associated with alterations in water balance that cannot be altogether disregarded.

MAYER: They cannot be disregarded, but I think it is possible both in experimental animals and in man to show that there is no great increase in the fat content of the body at that particular period of development.

WATERLOW: You would have to have a very large increase in body fat to upset Professor Terroine's calculation seriously, since the gain in protein is calculated as less than one fifth of the gain in body weight.

MAYER: In boys it is well known that an adolescent puts on a lot of muscle, and that the difference between the age of 15 and the age of 18 is largely a

difference in musculature; so I think that protein retention at that age is a very definite fact.

DEAN: In the European community it may be. I am not at all sure that it is quite so marked in a community of the kind that I am dealing with in Uganda. The pre-pubertal spurt in weight is not nearly so definite there as it is in European and North American children.

HOLT: Maybe that is their misfortune.

PLATT: On the contrary, it is generally agreed that the spurt is more marked in children who have been previously underfed; that is a fairly well-established fact, which Brody comments on in his classic book.[12] It is, in fact, a feature of Africa that such miserable, undernourished-looking children of 10 and 12 can bloom into such buxom maidens and such lusty-looking youths.[43] It is worth noting that in our Nyasaland survey the protein intake was found to increase markedly during adolescence (Table 31).

GOPALAN: We have made some growth studies in India, using vitamin B_{12} as a supplement, in the course of which we observed that the pre-pubertal spurt of growth was very significant, and could not be ignored. As far as Indian communities are concerned, I don't think I can confirm Dr. Dean's observations. As he has pointed out, the effect of endocrines on protein metabolism is something that needs further exploration. We do know, for example, that testosterone has a protein anabolic effect. With a given protein intake we may expect that there is better nitrogen retention when sexual function comes into its own. These are problems which I don't think have been fully explored. It is quite likely that at the time when there is an increase in growth, there is also a corresponding adjustment in nitrogen metabolism in the shape of better utilization of protein, about which we know very little.

MAYER: Dr. Holt made a very important point when he suggested that calculations of requirements should be referred to age of puberty or to some such developmental age, rather than to chronological age. There is plenty of evidence that the age of menarche varies from one area to another.

BENGOA: It is a statistical fact of considerable interest that all over the world mortality rates seem to be higher in boys than in girls, except in the period from 15 to 18 years, when the situation is reversed.

DEAN: Although adolescence seems to be a time when protein requirements are supposed to increase greatly, kwashiorkor in adolescents seems to be very rare. I don't know of a single report of the disease in an adolescent girl.

PLATT: They look after themselves very much better at about that time. The young men begin to eat with the men and get men's privileges, and the girls have special arrangements made for them. Both sexes get more food at that time in most simple communities that I know of.

GOPALAN: I don't believe that we have any really scientific evidence that adolescents do require increased amounts of protein. This may seem strange, but that is the impression I get. In the course of liver biopsy studies, one thing that strikes us is that fatty liver and the changes in the liver that we associate with protein deficiency are peculiarly rare in adolescents, even in these poor communities, and the picture of protein malnutrition has never been described in the pre-pubertal period; so here is a field for further study. I do not think we can say that simply because there is faster growth there is necessarily a greater need for protein.

TERROINE: I don't think we can invoke the absence of a fatty liver in adolescents as an indication that they have no greater need for protein. A fatty liver is not

only the result of protein deficiency; it is also due to a disequilibrium between the amounts of protein and of other foods absorbed. A fatty liver will develop with a high fat intake, even at reasonably good levels of dietary protein. But if both carbohydrates and lipids are low, then even though the protein intake is low as well, there is no disturbance of equilibrium, and a fatty liver will not appear in spite of the shortage of protein.

GOPALAN: I was not placing emphasis only on the fatty liver. Other changes associated with protein malnutrition are also peculiarly rare, in our experience, in that particular age group.

HOLT: To return to figures, is there an increase at the time of puberty in the NRC recommendations?

DARBY: There is no specific increase.

HOLT: I think there should be.

DARBY: There is only a statement that the protein intake should be adjusted to the increased calorie consumption.

CRUICKSHANK: Have any large-scale studies of protein intake been made?

HOLT: Yes, a study was made a number of years ago at Harvard, and the intakes did increase at the time of puberty.[11] This is the only one of the studies reviewed by Dr. Mayer[9] in which there was any increase.

DARBY: Similar studies have also been made by Ohlson and his colleagues.[45, 46, 47, 48]

DEAN: Dr. Widdowson's figures[25] do not show any increase in protein intake per kg. of body weight at puberty. I have intake figures for about a dozen adolescent boys; none of them had more than 60 g. of protein a day, of which 80 per cent was from vegetable sources, and they seemed to be healthy.

DARBY: But these are not minimal intakes, and that is where we get into differences of thinking. If you are talking about minimal standards, then, no doubt, from Professor Terroine's calculations, the protein intake should be increased, in order to maintain nitrogen equilibrium and to provide for growth.

HOLT: The minimum requirements do increase, particularly in the girl, at the time of puberty. For instance, Johnston[49] in a large number of nitrogen balances found striking deficits in adolescent girls. If the recommended requirements do not increase at the time of puberty, it obviously follows that the margin of safety which everybody wants to have is reduced.

DARBY: Except that we have a range for intake—from 1·5 to 2 g.—which gives a good deal of leeway.

HOLT: You have leeway and you are probably safe, but it seems to me very desirable to emphasize that there is an increased demand at that time, so that people will know they should give more to cover it. The exact figure isn't as important as the principle.

DARBY: I agree.

MAYER: Especially as in many societies the time of pre-puberty in girls is a time when restrictions are placed on activity, and therefore on calorie intake; so that if the protein intake is tied too closely to the calorie intake, they will tend to be short of protein at that period.

KING: Would you be satisfied to express the position as follows: it seems likely that there is an increased protein requirement at puberty, because of the increased rate of growth, but there is no clear-cut factual evidence to support this view?

HOLT: Are Dr. Johnston's negative nitrogen balances not to be regarded as clear-cut evidence?

DARBY: I think the balance studies of Johnston and of Macy are evidence. Johnston,[49] in summarizing his 20-year nutritional study of adolescent girls, noted several points pertinent to the present discussion. In children up to 12 years of age on mixed diets, consistently positive nitrogen balances were obtained only when the caloric requirement was met and when 15 per cent of the calories were derived from protein. Smaller proportions of calories from protein often resulted in negative balances. Johnston emphasizes that if the protein intake is to be adequate for the adolescent it must result in a retention of nitrogen and not merely in establishment of balance. In eight girls serving as normal controls in his series the nitrogen retained ranged from 22 to 105 mg. per kg. per day, or from 1·12 to 4·71 g. per day during the intensely anabolic phase at the menarche. This was on a nitrogen intake of some 12 to 15 g. per day (75–90 g. of protein).

Icie Macy[29] studied 29 children of both sexes from 4 to 12 years old during 593 balance periods. They were maintained on a good mixed diet. The averages of the nitrogen balances are shown in Table 33. The average retention in the 9–12 year old group is lower (0·80–1·05 g. per day) than in Johnston's subjects, although the intakes are approximately the same. This may be because both boys and girls were studied, and Macy did not restrict her observations to the highly anabolic period. Therefore the slightly lower figures are more applicable to the question of standards for groups.

TABLE 33

Averages of 593 average daily nitrogen balances for 29 children

Values are given in mg. per kg. body weight.

Age group	Intake		Output				Retention		Absorption
			Urine		Faeces				
year	mean	SD	mean	SD	mean	SD	mean	SD	mean
4	522·1	59·0	426·9	67·7	58·1	8·4	37·1	24·4	464·0
5	520·1	34·6	429·9	40·3	57·8	7·7	32·4	20·2	462·3
6	466·6	25·8	397·3	34·7	48·9	8·8	20·4	22·7	417·7
8	442·9	52·3	375·3	52·8	43·3	7·2	24·3	22·4	399·6
9	457·3	16·2	371·6	11·0	48·7	6·9	37·0	16·2	408·6
10	393·2	41·6	325·1	29·8	37·0	5·5	31·1	25·1	356·2
11	371·3	6·4	309·1	6·4	39·7	3·0	22·5	10·8	331·6
12	317·1	4·0	263·9	7·1	34·2	3·8	19·0	6·8	282·9

Taken from *Nutrition and Chemical Growth in Childhood* by I. Macy, 1942, Vol. I, p. 155, Table 55 by kind permission of Charles C. Thomas, Springfield, Illinois.

Other observers have recorded similar figures:—a nitrogen retention of 2 g. a day on intakes ranging from 11 to 23 g. nitrogen.[50, 51] Such findings have some bearing on the question of requirement, but none of them enables us to determine a minimum level that will permit normal growth and nitrogen retention.

KING: Although not everyone is in agreement, the balance of opinion seems to be that there is probably an increased need for protein at the time of adolescence because of the increased rate of growth. However, more data are needed before we can define the minimum requirement at this age.

Requirements of Adults

King: We have discussed the protein requirements of age groups in which growth can be used as the criterion of an adequate intake, and we must pass on now to consider the requirements of adults, and the criteria by which those requirements should be determined. We have already discussed some aspects of this problem in the first session, and we should try to relate those conclusions to the concrete problem of adult needs, not under experimental conditions, but under the stresses of ordinary life. It may well be that we cannot reach agreement either on a definite figure for adult requirements, or on the criteria which must form the basis for recommendations put forward. In that case, it will be useful to record our differences of opinion as an indication that further work is urgently needed.

As Dr. Darby has told us, all the bodies that have considered this problem put the adult requirement at about 1 g. of protein per kg. per day. May we have comments on that figure?

Waterlow: That figure includes a good proportion of animal protein. If we translate it into terms of a diet in which all the protein is from vegetable sources, presumably the figure would have to be substantially raised, say to 100 g. of protein for a 70 kg. man. Surely this is far greater than intakes actually observed in tropical countries? In other words, if we accept this figure we are in danger of striving for a target which cannot possibly be achieved in under-developed countries and in tropical dietaries. This is the point made at the very beginning by Dr. Aykroyd. In different ways it is as harmful to over-estimate as to under-estimate the requirements.

EXAMPLES OF PROTEIN INTAKES

Platt: It may be useful to give some figures of actual intakes. We have calculated the amino-acids supplied by three typical African diets evaluated at a 2,500 calorie level. The results are shown in Figure 19, in comparison with Dr. Rose's estimates of adult requirements. I am referring to his " minimum " level, not his "safe" level.

Diet 1, which was one used in a Nyasaland hill village, was based on maize-flour; diet 2, for a village near the lakeshore, was based on cassava, and diet 3, for an up-river village in the Gambia, on rice. Details of the composition of the diets are given in Appendix III. Without exception the diets, at the 2,500 calorie level, meet the minimum requirement for all the amino-acids.

Gyorgy: Were these entirely vegetable diets?

Platt: No; they are mainly vegetable, but sometimes there is a little fish.

The distribution of the protein between the various food categories for diets 1 and 2 is shown in Table 34 (p. 116).

The values for a, b and c for the lakeshore village are for three periods December–March, April–June and July–September respectively. Period a is in the rainy season, period c in the dry season. The effects on food supply of these seasonal conditions is reflected in the amount of protein from fresh fruits and vegetables.

In the cassava diet, with the lowest total protein intake, the limiting amino-acids are tryptophan, methionine and cystine.

Darby: The other two diets (1 and 3) supply about 1 g. of protein per kg., and yet they do not nearly attain Dr. Rose's *safe* level.

Holt: Were the people in good health?

Platt: Generally speaking, yes, in spite of parasites.

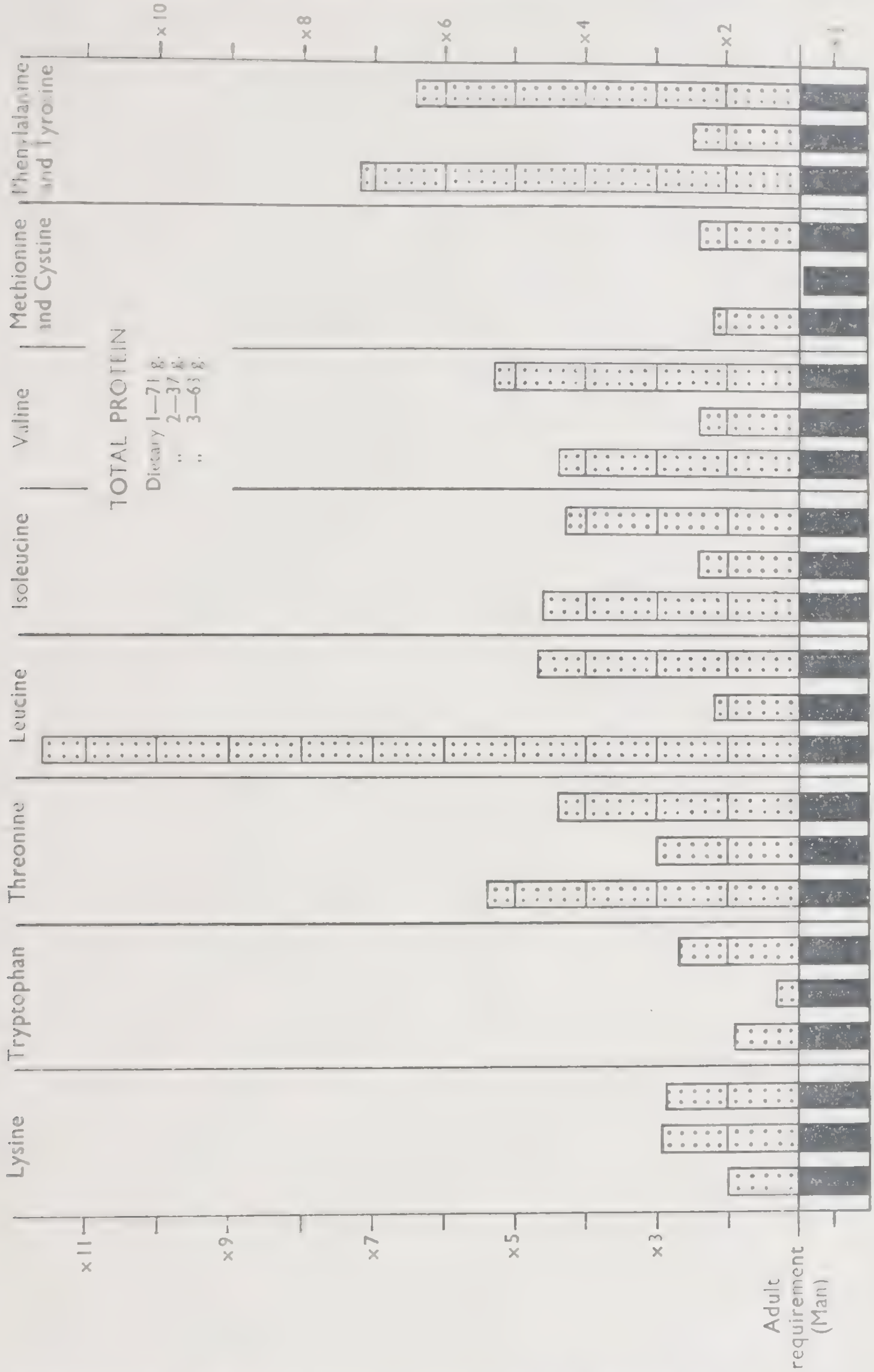

FIG. 19. Essential amino-acids supplied by three typical African dietaries at a 2,500 calorie level. (The following values were taken for the adult requirements (g. per day): lysine 0·8, tryptophan 0·3, threonine 0·5, leucine 1·1, isoleucine 0·7, valine 0·8, methionine plus cystine 1·1 phenylalanine plus tyrosine 1·1.)

SCRIMSHAW: We have some rather similar figures for the protein intake of families in the different Central American countries. If the intakes are adjusted for body weight, and compared with the NRC recommended allowances, the results are as follows:—

Costa Rica:	87 per cent of NRC allowance	
El Salvador:	86 ,, ,, ,, ,, ,,	
Guatemala:	130 ,, ,, ,, ,, ,,	(predominantly from corn)
Honduras:	117 ,, ,, ,, ,, ,,	
Panama:	108 ,, ,, ,, ,, ,,	

TABLE 34

The average intake of total dietary protein and amounts obtained from various categories of food in two African (Nyasaland) villages

District	Total protein (g.)	Source of protein (g. per head per day)					
		Cereals	Roots and tubers	Pulses	Fruit and vegetables	Animal (inc. fish)	Native beer
Hill village	50·3	31·7	0·4	8·8	3·3	4·0	2·1
Lakeshore village ..	25·1	1·0	5·0	2·6	3·0	13·0	0·5
Lakeshore village a ..	28·0	1·5	5·1	0·7	4·3	16·2	0·2
Lakeshore village b ..	23·7	1·1	4·9	5·8	3·0	8·2	0·7
Lakeshore village c ..	23·7	1·2	5·0	1·8	1·4	13·6	0·7

KING: But that was total protein, and doesn't give much indication of the amino-acid supply.

SCRIMSHAW: It was mainly vegetable protein. We have calculated the intakes of lysine, tryptophan and methionine. They are close to the safe allowances for tryptophan and methionine (even without taking cystine into account), and double the safe allowance for lysine.

KING: Yet you have protein deficiency in these countries.

SCRIMSHAW: We don't know that we have in adults. They are carrying on an active life, and are able to work hard.

GOPALAN: We have some data on the protein requirements of Indian male adults. Adult male volunteers in Coonoor were given various levels of protein, and nitrogen retention was plotted against nitrogen intake. We have taken only the positive balances because we believe—and I think the point is now generally conceded—that data based on negative balances may not be valid. Statistical analysis revealed a high degree of correlation between the nitrogen balance and nitrogen intake. From the graph we found that to maintain nitrogen equilibrium the required nitrogen intake was 3·55 g. per sq. m. of body surface. This works out at about 38·2 g. of protein per day for an adult weighing 70 kg. and height 5 ft. 6 in., or 0·5–0·6 g. of protein per kg.

GYORGY: If we translate Dr. Rose's amino-acid requirements into terms of protein, we reach a figure of about 0·5 g. per kg. This would be good quality protein, because Dr. Rose used an optimum mixture of amino-acids. The experiments done in Dr. Stare's laboratory[52] gave a figure of the same order — a requirement of only 30 g. of protein a day, even though most of it was of vegetable origin.

MAYER: The figure of 1 g. per kg. for the adult would not be consistent, I think, with 1·5 g. for the growing child; it would entail a much greater margin of safety, or difference between minimum and optimum.

GYORGY: Therefore I would rather recommend 0·5 g. per kg., always bearing in mind, of course, that we are talking about the ideal protein of Professor Terroine.

KING: Would that mean that you favour keeping the requirement at roughly 1·5 g. per kg. until the end of the growth period?

GYORGY: I would rather taper it off from 1·5 to 1, and then to 0·5 g. But that is a kind of geometrical extrapolation, and whether it is scientifically sound I am not prepared to say.

COMPARISON WITH ANIMAL REQUIREMENTS

KING: Perhaps those who have worked with animals could tell us what ratio they find between the requirements of the growing animal and of the adult. Then, applying this ratio to man, we might deduce a figure for the adult requirement, since we have agreed that in terms of Professor Terroine's ideal protein the child at 1 year needs 1·5 g. per kg.

ALLISON: I have some data of that kind on dogs, which may apply, relatively speaking, to the rat as well. I am assuming that ideal proteins are fed, such as those found in milk and eggs. The adult beagle dog needs about 70 calories and about 1·25 g. of protein per kg. body weight per day; that is, about 18 mg. of protein per calorie. On the basis of surface area, this comes to about 25 g. protein per sq. m. If we use this relationship between protein and calories, adult man would also require some 25 g. of protein per sq. m., or about 0·7 g. per kg.

In the puppy at the physiological age that corresponds to 10–12 years in man, it can be estimated that the protein requirement for maintenance and growth is about 3·2 g. per kg., or 25 mg. per calorie. In a very young dog, just weaned, about 6 weeks old, the requirement would be approximately 7·5 g. per kg., or 40 mg. per calorie.

CATRON: In broilers the optimum protein-calorie ratio seems to be about 44 mg. of protein per calorie.

ALLISON: The figures I mentioned show the relationship between calorie and protein intake for the production of *maximum nitrogen balance* at all these ages. The protein : calorie ratio is more than twice as high in the weanling puppy (40 mg. per calorie) as in the adult dog (18 mg. per calorie).

CRUICKSHANK: Yes, but on the basis of body weight the difference is much greater. Per kg., the protein requirement of the puppy at weaning is 6 times that of the adult—7·5 g. as opposed to 1·25 g. per kg. If we applied this ratio to man, and used the figure of 1·5 g. per kg. for a 1-year-old child, the adult requirement would come out at only 0·25 g. per kg.

TABLE 35

Composition of milk and rate of growth

Species	Days required to double birth weight	Composition of milk (g. per 100 g.)			
		Protein	Ash	Calcium	Phosphoric acid
Man	180	1·6	0·2	0·033	0·047
Horse	60	2·0	0·4	0·124	0·131
Cow	47	3·5	0·7	0·160	0·197
Goat	22	3·67	0·77	0·197	0·284
Sheep	15	4·88	0·84	0·245	0·293
Swine	14	5·21	0·81	0·249	0·308
Cat	9½	7·00	1·02	—	—
Dog	9	7·44	1·33	0·454	0·508
Rabbit	6	10·38	2·50	0·891	0·997

GYORGY: That argument is a fallacy, because a puppy grows faster than a human being and therefore has a higher requirement. Table 35, which was compiled by Freudenberg[58] from the old data of Bunge, Abderhalden and

Proscher, shows that the milk designed by nature for fast-growing animals provides larger quantities of protein, ash, calcium, and phosphoric acid—all indispensable building materials for tissue and bone formation. In general, fast-growing animals also are provided with a milk of higher caloric value.

GOPALAN: I am not sure that such comparisons are really valid. What is it you are trying to show by this table?

GYORGY: Only that the puppy has a higher protein requirement on account of its higher growth rate.

WATERLOW: The puppy must be laying down protein not only more rapidly, but also much more efficiently than the human infant; it must retain a much higher proportion of the nitrogen absorbed.

MAYER: Any species that grows slowly is automatically much less efficient, because the needs for maintenance are very much more important in proportion to the needs for growth.

WATERLOW: In other words we can't apply to man a ratio for adult to infant needs derived from the dog, because this ratio is necessarily different in the two species.

NITROGEN BALANCE AND PROTEIN STORES

MAYER: Logic has in fact caught up with us, because, as Dr. King indicated, we still have not defined our criteria. In talking about maintenance and adult requirements, in fact what we mean is the requirement for nitrogen balance. But at no time have we explicitly agreed that this is how we define the term "requirement". There isn't much point in recommending simply that people should be in nitrogen balance, because obviously if they aren't, they will die. We have to strive for a somewhat better target than the lowest possible nitrogen balance.

For protein, as for practically any other nutrient, one can be in balance over a large range of intakes; the difference lies in the stores that one wants to maintain. If one is in balance on an intake of 0·5 g. of protein per kg. per day, the chances are that the protein stores, particularly in the liver, are going to be somewhat lower than if one is in balance at 1 g. Thus, when we indicate at what level we want to establish balance, we are in effect choosing the type of stores that we consider to be minimal or optimal. I think there would be general agreement that above a certain level of protein intake there is no additional storage, or at least no additional storage of physiological value; but certainly there is a wide range in which there is a variation of stores with variation of intake. Therefore, from the physiological standpoint, we must be specific and clear about our criteria of requirement.

ROSE: Yes; we have to make clear, no matter what standard we use, whether we are talking about minimal safe intakes, or about desirable or optimal intakes. In adults there may be quite a difference, and we do not know how to measure the optimal. The values that I have chosen to refer to as safe intakes would not allow too much for regeneration purposes, if the individual were already depleted.

FREMONT-SMITH: As a compromise, I suggest this kind of formulation for the adult requirement: "the amount needed to maintain health and reasonable protein storage". In this way, it seems to me, we could make a recommendation that is fairly practical, and would be somewhere in between "minimal" and "optimal". This is a common-sense approach, a first approximation for countries or groups of people who are at the present time clearly deprived of an adequate

food intake. Gradually, we might raise the level towards the optimum, which in time we might be able to define more precisely.

TERROINE: I agree with Dr. Fremont-Smith's suggestion, but the main difficulty is this: we can, indeed, determine a certain figure for the minimum protein requirement of the adult as compared to the child, because we possess precise data in this field. I refer, of course, to measurements of nitrogen expenditures and losses. We know, in fact, exactly what to do to achieve nitrogen balance in the human body. I also entirely agree with Dr. Mayer and Dr. Holt that it would not be wise to fix the adult protein requirement at the minimum level necessary to maintain nitrogen balance. To build up a certain capital of labile protein might perhaps be regarded as a kind of insurance, enabling the organism to put up a better resistance against infections and stresses of various kinds. But here we are treading on unexplored ground, for we have no scientific facts which enable us to fix this *minimum for health*. Such a level would be distinctly higher than the *physiological minimum*, and might even be regarded as a *physiological optimum*.

HOLT: I should like to make a comment about protein stores. It is perfectly clear that by increasing protein intakes one can increase protein retention. That protein is going somewhere, perhaps to the liver and kidney in particular. But to speak about it as a store implies that it has value in times of stress. But is there any evidence for this? Dr. Warren Cox investigated the point in some experiments which have not been published. He forced rats to gain weight in the form of liver and kidney tissue, and then deprived them of protein and calories to see if they survived any longer than control rats. They did not; they died at just about the same time. Therefore I think we are deluding ourselves when we talk about stores as if they were necessarily valuable.

MAYER: How, then, do you explain your own results? Unless there is some sort of protein store somewhere, how is it that when you deprived a child of phenylalanine, it did not go into negative nitrogen balance, but just stayed in balance and stopped growing? (See Figure 3, p. 20.) Obviously there must have been a rearrangement of amino-acids from some other source in the body; otherwise, in the absence of any protein store, there would have been negative balance.

HOLT: I assume that tissues of different composition were changing, one tissue being destroyed and another tissue being constructed.

MAYER: But isn't that the equivalent of some reserve?

HOLT: Not necessarily reserve. We don't know what it is. I don't see the virtue of giving a high protein diet just to get a great big liver and a great big kidney.

GYORGY: I have an unpublished interim report from the Medical Nutrition Laboratory of the Army, from the group led by Dr. Grossman. They have fed protein to rats in amounts one, two or three times the minimum requirements, with calories sufficient to maintain their body weight, and subjected them to burns or to surgical stress in the form of laparotomy. After the stress negative nitrogen balance was most marked in those rats which had previously received twice or three times their normal requirement, but there was absolutely no effect on the healing of the laparotomy or burn. I would like to quote their conclusion verbatim:

"Maintenance of a high plane of protein nutrition before injury is without obvious benefit to the animal as compared with minimal adequate nutrition."

WATERLOW: I think Dr. Holt's thesis is very dangerous. I entirely agree that more experimental work is needed on this problem, but in practice we are not

concerned with amounts of protein two, three, or four times the optimal. The fact is that in tropical and under-developed countries we do see people with atrophy and protein depletion of many organs. In infants we have found livers whose total protein content was 40 per cent or more below normal by American or European standards.[54, 55] I think it is legitimate for us to assume that this is bad, rather than to accept the onus of proving that it is bad. I admit that I can't prove it, and I hope experimental work will be done on this subject, but I would not like your thesis to be widely accepted.

HOLT: You misinterpreted my thesis. I am not arguing that a deficiency is good, only that it remains to be shown that an excess is valuable.

WATERLOW: I wasn't talking about an excess, but about the difference between a minimal plane and something above a minimal plane.

HOLT: I am not arguing for maintenance of a bare minimum. I don't think anybody could. Obviously there has to be a margin of safety.

WATERLOW: Haven't you shifted your ground, then? In the experiments you quoted the rats were force-fed and therefore presumably got a large excess of protein. In the experiments at the Medical Nutrition Laboratory the rats were given "two or three times their normal requirement". These experiments are therefore irrelevant to the point at issue, which was that it is important physiologically to have an intake that will maintain good stores.

ALLISON: I have some experimental data that may be helpful. The protein stores in the dog increase, if the nitrogen intake is raised from 2 to 4 g. nitrogen per sq. m. of body surface area per day. Dogs maintained in nitrogen equilibrium on intakes varying between 2 and 4 g. nitrogen per sq. m. per day are often considered normal. There is, however, an upper limit to these stores, so that when a certain level of activity of xanthine oxidase or of other enzyme systems in the liver is reached, it doesn't do much good to increase protein intake further. Under conditions of stress, such as are produced by a toxic drug, resistance may be reduced in the animal fed at the lower level, even though the animal be considered normal and in nitrogen equilibrium. For instance, the phosphoramides produced a marked leucopenia within 2 weeks in animals fed the lower nitrogen intake. However, if the dog was fed more nitrogen, which increased the protein stores, but still within the so-called normal range, then the leucopenia did not develop.

An intake of less than 2 g. nitrogen per sq. m. per day produced a dog that was obviously depleted. Once you get into the depleted range, fibroblast formation and the healing of wounds are much slower, the toxicity of the phosphoramide is much greater, and the ability to resist calorie deficiency is much less than in the normal rat or dog, even though the animals are in nitrogen balance. Therefore we do have a safety factor, in the variable protein stores.

GYORGY: Neither Dr. Holt nor I proposed a deficient diet, and we are in full agreement that there should be a margin.

WATERLOW: Dr. Holt said, "What evidence is there that it is better to have stores than no stores?" I think Dr. Allison has answered that.

DARBY: Perhaps we have reached a point where it may be useful to summarize what has been said about intake levels and requirements of adults. There is good evidence that nitrogen equilibrium can be maintained on intakes of about 0·5 g. per kg. per day of a good-quality protein. Dr. Mayer and Dr. Davis in their paper[9] have summarized this evidence, including re-investigation of the problem in Dr. Stare's laboratory.[52] Indeed, in that study it was found that equilibrium could be maintained on intakes even lower than 0·5 g. per kg. This

then, might be one minimum, not necessarily *the* minimum. Then, as we go upward from that level, a number of people working with proteins of different composition have suggested 0·7 and 0·8 g. per kg. as standards of intake. Finally, when we come to levels of intake which seem to be about maximal, or at least which are clearly associated with excellent health, we reach a figure of about 1 g. per kg., assuming an intake of mixed protein. However, for us to select any one of these values would be impossible.

The work described by Dr. Allison has raised doubts in many of our minds about the validity of our criteria. The minimum intake necessary to secure nitrogen balance may be a physiological entity like the basal metabolic rate; but would it be wise to equate this with what Professor Terroine has called the "requirement for health"? Probably not. Unfortunately, once we go above that minimum level, we have no means of knowing how large a safety margin to allow. At the moment all we can do is to stress this very serious gap in our knowledge.

Requirements during Pregnancy and Lactation

EXISTING RECOMMENDATIONS FOR PREGNANT WOMEN

DARBY: There have been a number of studies in recent years in the United States on the dietary intakes of women during pregnancy. In Tennessee the intake of protein in a group of women who had satisfactory health during pregnancy was on the average about 75 g. per day, varying a little according to trimester.

Dr. Scrimshaw told me that a summary of his data from Rochester has revealed a very similar intake level, within a gram or so. Furthermore, in both these studies the incidence of those accidents of pregnancy which are in essence unavoidable was comparable to that which one would find in the national averages; so that this, perhaps, will give us a measure of the level at which we can find good health performance during pregnancy. I am not proposing this either as a minimal or as a maximal intake; I am merely citing it as an experience.

The Food and Nutrition Board recommendation is that during pregnancy the protein intake be raised to 80 g. per day in the third trimester. This is assuming a non-pregnant weight of about 55 kg., and a non-pregnant protein allowance of 55 g. per day, so that the increase is nearly 50 per cent. The Canadian standard is very similar; they add 25 g. per day to the normal allowance.

KING: But did you actually find so large an increase in the protein intake?

DARBY: No, because the caloric intake does not rise as much as has been estimated.

KING: Dr. Stuart and his associates at Harvard found that the protein intake of the mothers they studied was correlated with their health during pregnancy and with the health of their offspring.[56]

HOLT: I wonder if there aren't other variables in that Boston study—economic status, better care, and all kinds of other things?

KING: There probably were: that question often comes up. However, these results tend to justify the higher protein intakes during pregnancy.

In the studies made in Detroit, Dr. Hoobler[57] found increased protein storage in the mother with the establishment of pregnancy and then with the development of the foetus. As far as I know, that is one of the most careful studies that has yet been reported of a fairly large number of normal women who were under medical supervision while biochemical tests were done.

REDUCTION OF ACTIVITY DURING PREGNANCY

HOLT: I am sure that was a very careful study, but I would like to bring out another point of view that the additional demands caused by the child are minimal during pregnancy. If you add together the child and the fluids and the increase in breast tissue, etc., the total increase in the amount of protoplasm to be nourished probably amounts to about 20 lb. in all. The energy demands, are, however, less, because the average pregnant mother is less active: as far as calories go, these two factors compensate for each other and there should be no increase in calorie requirement. There is no basis for the advice which was often given to the mother to "eat for two".

DARBY: Our results support that.[58] We found that the woman during the first trimester of pregnancy had an average intake of 2,140 calories; in the second trimester, 2,200 calories; and in the third trimester 2,020. In other words, during the third trimester, in our pattern of life, there is apparently a decrease in activity which is reflected in decreased calorie intake. In our survey the average weight gain during the pregnancy was about 20 lb., which is conceded by the obstetricians to be the ideal. This therefore supports your statement that there is little need for an increased energy intake in pregnancy, provided, of course, that activity is decreased. This may not apply when the mother has to continue hard physical labour, going a couple of miles for water, working in the fields, etc., until the day of delivery. I am not sure, therefore, that we can generalize these results for the rest of the world.

SCRIMSHAW: In Rochester, New York, the average weight gains in 500 consecutive clinic pregnancies, and in 500 consecutive pregnancies in women attended by private physicians, were 25 and 24 lb. respectively. These results are essentially identical with those cited by Dr. Darby. The increase in calorie intake and the increase in total protein intake with gestation were not statistically significant.

MAYER: I very much agree with what Dr. Darby and Dr. Scrimshaw said about what amounts to an over-emphasis in Western countries on increased requirements during pregnancy, at a time when decreased activity accounts for at least the 400 extra calories recommended, for instance, by the National Research Council. I think there is no factual basis for increasing the requirements.

DEAN: Have you a factual basis for the decrease in activity?

MAYER: Yes. We have already heard that in pregnancy women have put on the normal 20 lb. in weight, with no increase in intake; moreover, in studies in Boston, obese pregnant women have been successfully reduced in weight while pregnant, with no damage whatsoever to the child. But all this assumes that the woman were adequately fed in the first place.

NECESSITY FOR INCREASED INTAKE

WATERLOW: One can calculate that of the 20 lb. which a woman puts on during pregnancy, about 3–4 lb. consists of protein. This means that about 5 g. of protein are laid down per day, and if one assumes an efficiency of conversion of 50 per cent, the mother would need an extra intake of 10 g. per day. If she has already been getting 70 g., she has a margin and doesn't need any more; but if she has only been getting 40 or 50 g., she may well need that extra 10 g. Therefore I feel that the discussion so far has been a little on the incautious side.

TERROINE: If on an intake of 70–80 g. a day no increase is necessary during pregnancy, we can only conclude that that intake was much too high to start

with. We must remember that during gestation there is not only the production of the foetus and adnexa, but also a significant increase in the mother's protein stores, resulting from the action of the sex hormones.*

I have calculated that about 1 g. of nitrogen a day, or 6–7 g. of protein, are needed for the formation of the foetus and adnexa, and to provide for this increase in the protein reserve of the mother. Therefore, we must conclude that if in countries such as the United States or the United Kingdom or France, the daily intake of protein is high to start with, then, perhaps, it is not really essential to increase that intake during pregnancy. In the under-developed countries on the other hand, where the initial intake is low, it is quite essential to increase it.

DARBY: Dr. Icie Macy Hoobler, in the nitrogen balance studies to which Dr. King has already referred, concluded that the protein intake should be increased by something like 20 per cent to cover the added requirements of pregnancy.[57] That is not very different from the increase of some 7 g. a day proposed by Dr. Terroine and Dr. Waterlow. The recommendation of the Technical Commission of the Health Committee of the League of Nations[57] for the protein allowance during pregnancy was 1 g. per kg. per day for the first trimester, and 1·5 g. per kg. per day for the period 4 to 9 months.

KING: Does everyone agree that the increase in protein requirement during pregnancy might be in the range of 10 g. per day; that this is a reasonably safe figure, for which there is some evidence?

PLATT: We should add that the increase is needed particularly towards the end of pregnancy. In a recent review by Garry and Wood[59] it is concluded that an intake of 2 g. per kg. of body weight is probably justified toward the end of pregnancy, but is unnecessarily high in the early stages.†

DARBY: I think that would be unnecessarily high even at the end, from our own experience. It has been suggested that the increased intake is needed in preparation for lactation. In the series of pregnant women studied by us 71 per cent " lactated ", by which I mean they nursed their babies. This is a reasonably high proportion, judged by experience in the United States. Yet their mean protein intake during pregnancy had not been more than 70–75 g.

KING: Well, do you think that 10 g. is a reasonable extra allowance?

SCRIMSHAW: Yes, if the woman was previously on a minimum intake.

DEAN: I agree. Professor Terroine's point is important. If you have an intake of 70 g. of protein, you probably don't need an extra 10 g., but if the intake is only 40 g., you undoubtedly do need the increase.

DARBY: I think that for guidance in supplementary feeding programmes we might emphasize that above a certain level of intake—and I don't think we can fix that level more definitely than at about 70–80 g. of protein a day—there does not seem to be any benefit from increasing the allowance of protein. This, perhaps, places a ceiling on levels we should aim at in protein-deficient areas.

AYKROYD: There are now a considerable number of supplementary feeding programmes throughout the world benefiting pregnant and lactating women.

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* The nature of these hormones and the mechanism of their action are being studied in France by Mlle Bourdel, under the direction of M. Jacquot. (E. Terroine.)

† Garry and Wood[59] point out that "there has been a tendency to advocate a somewhat smaller intake of protein during pregnancy than was previously considered necessary". It is worth noting also that "Coons and Blunt from their study of lactation in women make the significant remark that the greater the nitrogen retention, especially in the latter part of pregnancy, the more likely is lactation to be adequate".[60]

and the question of how much of a food such as skimmed milk should be supplied to these women is certainly of practical importance.

CRUICKSHANK: The same criticism could be levelled at the figures we have heard as was made of Dr. Widdowson's, when we were discussing the requirements of children: that they merely calculated from the known intakes of healthy women during pregnancy. If we are looking for minimal figures, are there any data available from other areas showing a much smaller intake of protein by a woman who is healthy throughout pregnancy and has a healthy child at the end of it?

SCRIMSHAW: In 1946–48 we studied the dietary intakes of a group of pregnant women in Panama.[61] Low-income Panamanians averaged 50 g. of protein a day, with a range of 26 to 104 g.; West Indian negroes living in Panama averaged 47 g. with a range of 27 to 77 g. Fifty women were sampled in each of these groups. About one third of the protein was of animal origin. The incidence of toxic complications among these women was no higher than in groups in the Northern United States with considerably higher protein intakes. Furthermore, the protein intakes of women actually developing pre-eclampsia showed approximately the same mean and standard deviation as those with an uneventful pre-natal course. In general, their pregnancies can be said to have been satisfactory, but it was not possible to follow the children born of these mothers.

FREMONT-SMITH: Do we have any kind of a level that we know for certain is not quite enough?

GOPALAN: In pregnant women in a community which we have been investigating, the protein intake was between 40 and 45 g.; that was definitely inadequate, in the sense that a large number showed anaemia and low serum protein levels.

CRUICKSHANK: Were they anaemic before they became pregnant?

TABLE 36

Signs of dietary deficiency in 198 *pregnant women in South India*

Average intake per day: calories 1,211, protein 35 g.

Sign	Percentage incidence
Oedema	9·5
Vitamin A deficiency	14·5
Vitamin B complex deficiency	44
Burning feet syndrome	12
Haemoglobin concentration:	
<10 g. per 100 ml.	56
10–12 g. per 100 ml.	29
>12 g. per 100 ml.	15
Total plasma protein concentration:	
<5 g. per 100 ml.	7
5–6 g. per 100 ml.	24
6–7 g. per 100 ml.	21
>7 g. per 100 ml.	48

GOPALAN: This was a cross-sectional study. We saw the women at a given point of time: at that time a considerable proportion had low serum protein levels and only 15 per cent had anything like a normal level of haemoglobin. Most of them had stomatitis and B complex deficiency. These findings, together with those from a control group, are shown in Table 36. The incidence of pre-eclamptic toxaemia was the same in the two groups.

GYORGY: What kind of protein were the poor-class women getting?

GOPALAN: Predominantly vegetable protein—cereals and pulses. I expect that their calorie intake was about 2,000 per day.

BIRTH WEIGHT OF INFANTS

FREMONT-SMITH: What was the condition of the infants?

GOPALAN: We made some measurements of birth weight. The results are given in Table 37. The incidence of immaturity was eight times higher in the group on the poor diet compared with the group on the better diet.

TABLE 37

Frequency distribution of birth weights in South India

Subjects	Sex	No. of births	Per cent of babies weighing					
			3–4 lb.	4–5 lb.	5–6 lb.	6–7 lb.	7–8 lb.	>8 lb.
Upper classs.. ..	M.	100	0	1	6	29	37	27
	F.	100	0	3	10	38	33	16
Lower classs.. ..	M.	190	3	7	28	36	18	8
	F.	250	1	8	35	37	16	3
Cases of kwashiorkor	M.	16	0	12·5	25	50	12·5	0
	F.	18	5·5	11	5·5	55	22	0

VERHOESTRAETE: For comparison with Dr. Gopalan's figures, if we take the internationally accepted standard of 2,500 g. as the weight below which a baby is considered premature, the prematurity rate in the United States is 7–8 per cent, and in Chile 12 per cent.

GOPALAN: That is the criterion I used. By this standard nearly 50 per cent of the lower class children would fall in the premature group. However, immature is perhaps a better word, since I am judging by weight and not by time of gestation.

PLATT: I would call them full-term but immature, not premature by Western standards.

DEAN: Many children born in Uganda are certainly below the international weight standard, but I am not at all sure that they are immature. Dr. Greulich[62] has some evidence that he can judge the development of a premature child by the bone age. I think it might well be that these light-weight children are perfectly normal, but in the last few weeks of pregnancy did not put on the large amount of fat which is usually put on by European children. There might be a racial difference, in this respect.

GOPALAN: Table 37 also shows some figures for the birth weights of children who subsequently developed kwashiorkor. This group is admittedly very small.

but it is interesting that the weights are no lower than in the poor group as a whole. Therefore, although there is definite evidence that protein malnutrition in the mother is a factor affecting the birth weights of the infants and probably their neonatal status also, I don't think it is a factor directly related to the development of kwashiorkor.

DEAN: Would you agree that these children who are light in weight do not behave in any way like premature children, as seen in England? Do you think that these children are under any disadvantage because of their low birth weight?

GOPALAN: No. The Indian obstetrician uses 5 lb. (2,270 g.) as the criterion. These children between 5 and 5·5 lb. do not need the special care and nursing which are required for premature infants. I think the distinction between premature and immature which Professor Platt raised is important.

PLATT: When we know more about them I suspect we will find that they are not as well and fully developed as they should be.

LACTATION

PLATT (continued): The important point, I think, is that an increase in the diet towards the end of pregnancy should be regarded as preparation for successful lactation. I think that is more to the point, perhaps, than finishing off the foetus.

FREMONT-SMITH: Is there any practical experience in support of the view that a moderately malnourished woman can sustain pregnancy better than she can sustain lactation? In other words, that she can more easily provide an adequate foetus than sustain the growth and development of the baby?*

DARBY: I think, as Dr. Platt said, that lactation is a more stressful period than pregnancy, in so far as meeting the nutritional needs of the mother is concerned, because she really is feeding one for two. Physicians and research workers have given too little attention to maternal requirements during this period.

TERROINE: I am very glad to hear Dr. Darby say that, because I pointed out many years ago, in the League of Nations report,[64] that too much stress was being laid on protein requirements during pregnancy, while it is the total requirements during lactation that we should, in fact, consider.

MAYER: There is, I think, a considerable jump in requirement at the time of lactation. From the nutritional standpoint, this is a point at which women are very much neglected, in the United States and elsewhere. The mother has been much subjected to propaganda about nutrition in pregnancy and so little about requirements in lactation that at a time when her activity is again very high, she very often does not feed herself well.

KING: Dr. Darby, can you give us the published recommendations for the period of lactation?

DARBY: The requirements for lactation are generally conceded to be higher than for pregnancy. In the League of Nations standard[37] the level of 2 g. per kg. was proposed. The Food and Nutrition Board[23] recommended increasing the calorie allowance by about 1,000, and the protein allowance to about

* Some results obtained by Dr. Marianne Goettsch in the rat are of interest here.[63] Rats were fed a rice and beans diet at various levels of protein intake. The following is a summary of the most important results:

Protein of diet (per cent)	24·8	16·7	11·9
Mean birth wt. of young (g.)	5·45	5·35	5·3
Mean body weight of young at weaning at 21 days (g.)	35·0	33·8	28·5

At lower protein intakes all the young born alive died before the 4th day. (J. Waterlow.)

100 g. per day. This is for a woman weighing 55 kg. when not pregnant, so that their recommended allowance also approaches the level of 2 g. per kg. per day.

Unfortunately, in the Vanderbilt study[65] the data on lactation are less complete than those on pregnancy. We have some figures on the intakes of women during lactation; they are not the same group as those we studied during pregnancy, but they come from the same region. The protein intake during lactation averaged about 75 g. per day, rather than the 100 g. which is the recommended allowance: in other words, there was no appreciable increase over the intake during pregnancy. However, the calorie intake of lactating women in the southern part of our country is about 350–400 calories higher than that of non-pregnant, non-lactating women of the same age in the same population.

King: How great is the spontaneous increase in protein intake?

Darby: The protein intake increases with the calories; it went up from 58 to 75 g. in one group, and from 66 to 72 g. in another group.

Dean: Have you any idea how much milk these women were producing?

Darby: No, except that they were nursing their babies, and the babies looked well.

Platt: There are data for the United States, but, strangely enough, only for women who commercialize their milk production. Some of the older work showed that women who went on producing as much as 1½–3 litres of milk daily for a year or so took in of their own accord 150 to 160 g. of protein a day, chiefly as dairy products.[66, 67, 68] Budin, who is one of the few people who made any record over a year or more for European women, also measured the milk output of wet nurses, and, again, found a high level of lactation was maintained.[35]

Gopalan: The difficulty is that lactation performance is very often affected by the psyche. As Dr. Dean rightly pointed out, it is the women of the poorest socio-economic groups that lactate well, while the better economically situated members of the community seem to be unable to feed their babies. Therefore you cannot expect any direct correlation between protein intake and lactation performance.

Dean: I think that it is almost impossible to learn anything about the protein needs of the lactating mother from a highly developed society such as that of North America, and that we shall learn only by careful studies of people in under-developed countries, where it may be possible to show the effect of a small alteration in protein intake. There is no doubt at all that if you take American mothers and feed them twice as much protein, they will not produce any more milk. They might produce much less.

Mayer: Though psychological factors are obviously important, the thermodynamics must be the same in all. If we know the milk output, and if we know the conversion factor of food protein into milk protein, then we can easily calculate the extra requirement.

Gopalan: In Table 26 (p. 84) I gave some data on the output of breast milk of poor women in South India. The table also shows that the protein intake of these mothers ranged from 44 to 57 g. daily and the calorie intake from 1,300 to 2,400; the 24-hour milk output varied from 21 oz. in the first stage of lactation to 12 oz. in the 18 to 24-month period.

Gyorgy: Were the mothers getting animal or vegetable protein?

Gopalan: It was mainly derived from cereals, and to a certain extent from pulses. Animal protein does not figure to any appreciable extent in the diets of these women; they get it a few times a year only.

DARBY: What cereal?

GOPALAN: Rice.

DEAN: How do you measure the milk production?

GOPALAN: The mothers were admitted to hospital and the children were weighed before and after each feed throughout the 24 hours.

WATERLOW: The figures for 24-hour milk output that we got in Gambia were very much the same and we used the same method as you.

Why is the calorie intake of your mothers so much higher in the first 6 months?

GOPALAN: I deliberately withheld comment on that. Why should the calorie and protein intakes in these mothers progressively diminish with advancing stages of lactation? Are these observations really significant? I do not know. I do not want to read too much into these figures. They are based on 100 mothers, and perhaps the trend would be different when more are studied.

AUTRET: These are still active women?

GOPALAN: Yes, quite active.

SCRIMSHAW: Are the women the same at each period?

GOPALAN: No, this is a cross-sectional and not a longitudinal study.

MAYER: You don't think the facts of admitting them has changed their feeding habits?

GOPALAN: I don't think so. If there were any psychological factors, I don't think they were very important. It may well be that in response to post-natal care and advice these mothers tend to take more food during the early stages of lactation. That is one possibility. Another is that the diminution in the calorie and protein intakes in the later stages may be the result of the demands of the growing infant for supplementary feeds. I don't know what the exact explanation is, but I think the first is the more likely.

SÉNÉCAL: I don't understand your figures at all; I can't see how a woman could stay alive on an intake as low as 1,400 calories, taking into account the extra energy required for the production of milk.

GOPALAN: Yet they seem to be active, and are working on plantations. I am just putting forth data, and it is for the fundamentalists here to provide the explanation. In the course of nutrition surveys in undernourished communities, the intriguing thing is not that there is so much malnutrition but that there is so little of it, considering that the diets are so inadequate.

AYKROYD: Some of the calorie figures for Indian women have always been surprisingly low.

MAYER: Surely we ought to relate the increase in protein requirement during lactation to the amount of protein put out in milk. A simple calculation shows that the output of protein, calcium, etc., in milk is much greater than the extra amounts needed in pregnancy, and when lactation is prolonged for 6 months or a year the appetite often may not be able to keep up with milk output. Therefore the problem in countries where women have been poorly fed and lactate for long periods is different from the situation seen in the West.

DEAN: Everyone seems to be agreed that a great many of the women in the populations that we are dealing with are slightly undernourished, but it is the rule for these women to be remarkably successful in lactation—very much more so than the overfed women of the West.

MAYER: I was not suggesting that lactation was necessarily inefficient. All I was suggesting is something that is quite obvious in poor countries; namely, that women who have been subjected to repeated pregnancies and lactations age extraordinarily fast and are usually, at the end, in a poor state of nutrition. This also

may affect the child. In Calcutta, for instance, the paediatricians find that it takes a longer and longer time for newborn children to regain their birth weight as the number in the family increases. It could be 10 days, 20 days, or even 30 days, compared with 5 or 6 days in the West. This they think depends on the degree of malnutrition of the mother, probably as a direct result of anaemia, rather than of a failure of milk production.

DARBY: In lactation, as in pregnancy, the increase in protein needed must depend on the initial intake. I think that we might get perfectly good lactation at 70 g. per day, as indeed, Dr. Gopalan's figures would suggest.

DEAN: Has anyone any other information suggesting that women, who have previously had a low level of protein intake, spontaneously put up their intakes during lactation?

PLATT: It is a custom in China for the poorest of people to eat chickens, soup, eggs, etc., during pregnancy and lactation.

DEAN: That is so in at least one part of Africa, but not in most of the places about which I have information.

PLATT: It used to be the custom in one part of Nigeria until recently to put the girls into what was called a "fatting house" before they were mated and, if they could afford it, they went back again when they were feeding their babies.

EFFICIENCY OF LACTATION

PLATT (continued): To return to the point you made, that we should relate the increased requirement during lactation to the protein output in milk: have we any ground for disbelieving the old-established figure; that we need 2 g. of protein by mouth to make 1 g. of protein by breast?

MAYER: Dr. Leitch made some calculations for the first FAO Committee on Calorie Requirements.[24] She found an efficiency of lactation of about 50 per cent, which agrees with Dr. Platt's figure.

TERROINE: I do not know of any work done in France on the efficiency of protein production in human milk. It is possible that studies of this kind have been made in the cow, but I am not sure. In any case, it is not an easy problem to solve experimentally.

MAYNARD: With dairy cows the amount of protein needed for milk production over and above the maintenance allowance is about 35 per cent in excess of the amount secreted in the milk.* In other words, after allowing for maintenance, the conversion efficiency is about 75 per cent. Irrespective of the biological value of the protein as fed, the value of the amino-acid mixture absorbed is high as a result of rumen action.

DEAN: It is hardly fair to compare the cow with the woman. Dr. Maynard is dealing with an animal that has been bred entirely for its milk yield.

PLATT: The pig would be nearer the mark.

CATRON: Sows vary tremendously in milk production. The best figure I can give, for pigs given pituitary hormone, is 3 to 5 g. of crude feed protein per g. of milk protein. This is not taking maintenance into account. The maintenance requirement is about two thirds of this; if that is subtracted, we get a conversion ratio of almost 1 g. per g.

KING: It seems to me that the human data should have preference over the animal data, because of the great difference in the physiology of the cow and the pig.

* This figure is calculated on the basis of a maintenance allowance of 0·6 lb. digestible protein per 1,000 lb. body weight.

CRUICKSHANK: In fact, however, there is quite good agreement. The mother is not likely to produce more than 10 g. of milk protein a day (1 litre containing 1 per cent protein). Usually the output will be less, say 7 g. If we accept Dr. Leitch's conversion factor, this would need an extra intake of about 15 g. protein a day, which is very much in line with what Dr. Darby actually observed.

DARBY: Yes: however, that increase is much less than the NRC recommendation, according to which a woman weighing 55 kg. should increase her intake from 55 g. when not pregnant to 100 g. during lactation. It seems that this recommendation again is on the high side.

KING: Could we conclude that if we take the amount of protein being provided each day in the mother's milk, and multiply by 2 because of the physiological conversion factor, this would give the increase in intake necessary to maintain lactation?

DARBY: That is very reasonable.

PLATT: Before we end, I would like to re-emphasize and bring into better perspective, the importance of prolonging the *duration* of lactation. We should aim at feeding mothers well enough, to enable them to continue to produce an adequate volume of milk for a longer time than is normally accepted as breast-feeding practice in the more sophisticated countries.

Summary

In this second session an attempt was made to apply the principles and experimental results of section I to the practical problem of human protein requirements at different ages. Although it may be conceded that in principle these requirements can be formulated in terms of amino-acids, to do this for every age and sex would be difficult and cumbersome. As *Terroine* pointed out, a *reference* protein is needed, and for this purpose he proposed the mixed proteins of milk. Ideally, a conversion factor could then be calculated or experimentally determined for any other protein mixture.

The discussion began with the requirements of infants and young children. Here the use of milk as a reference protein is obviously appropriate. The criterion by which an intake was judged to be adequate was its ability to support 'normal' or 'satisfactory' growth. In practice this means gain in weight. The weakness of this criterion has already been discussed in section I; nevertheless, for general purposes it serves as a rough and ready guide.

The question of the protein needs of the growing infant can be approached from both ends, by working downwards from amounts that are known to be enough, or by working upwards from amounts that are certainly not enough. The first approach was followed in section I, and it was agreed, on the basis of Holt's experiments and of the observed intakes of normal breast-fed infants, that at 6 months the requirement in terms of milk protein is about 1·5 g. per kg. This figure provides one fixed point. In section II attention was concentrated on the second approach.

Fremont-Smith posed the important question: what is the highest protein intake that is known to be inadequate? The conference could not give any precise answer, partly from lack of data, and partly because it is difficult in practice to apply Terroine's conversion factor and to evaluate recorded intakes in terms of milk proteins.

Evidence from India, Africa and Central America suggests that an average intake for an infant of 1–2 years might be 20–30 g. of protein a day, mainly of vegetable origin. In these babies the rate of growth tends to fall off during the second year of life, or even earlier, and to improve if supplementary protein, such as skimmed milk, is given. This suggests that they are not getting enough protein, even though the intake in relation to body weight is between 2 and 3 g. per kg. per day. This method of expression is, however, misleading, because the body weight is too low.

It is known from Dean's work that satisfactory growth can occur on a protein intake entirely from vegetable sources of the order of 4 g. per kg. per day. This is a very high figure; moreover, such large intakes might bring with them the danger of amino-acid imbalance already discussed – a danger that may easily arise with diets based, for instance, on corn.

One outcome of the discussion was to emphasize the value of even small amounts of breast milk in helping to balance the protein mixture during the phase of rapid growth and high requirements. From this follows the importance of prolonging lactation for as long as possible. The nutrition of the child is therefore closely bound up with that of its mother. The evidence, although fragmentary, suggests that when the mother is undernourished the protein content of the milk is maintained, but its volume is reduced. There is little doubt that after 6 months the amount of breast milk is often too low to supply

all the baby's needs for protein. It is therefore not surprising that kwashiorkor should occasionally occur in infants who are still being breast-fed.

Although it was not strictly relevant to the problem of protein requirements, attention was given to some features of kwashiorkor that have not been much considered in previous discussions. It now tends to be assumed, implicitly or explicitly, that kwashiorkor, or the milder phase that some call pre-kwashiorkor, is diagnostic of protein deficiency. This may well be justified; nevertheless, the possibility of other deficiencies cannot be ignored. *Hansen* presented results from South Africa, showing that in kwashiorkor there may be a depletion of potassium which is even greater than that of nitrogen. Since potassium and nitrogen go hand in hand in all animal and plant tissues, this association is to be expected. It is possible, however, that potassium loss may contribute to some of the features of kwashiorkor, such as oedema.

In the treatment of kwashiorkor, good results have been claimed when very large amounts of protein are given (up to 100 g. a day). When this is done, there tends to be a temporary rise in blood urea. The question arose, what is the rationale of this high protein therapy, and may the results not be harmful? *Platt* produced experimental evidence that refeeding after depletion may indeed cause damage to organs that were apparently unaffected by malnutrition *per se*, particularly if the process is repeated through several cycles. A recurrent stress of this kind may occur in tropical countries, where there are alternating seasons of hunger and plenty. This is an important conception; moreover, it emphasizes the fact that, although kwashiorkor appears in its most dramatic form in the period immediately after weaning, the conditions that produce it may operate in greater or lesser degree throughout childhood.

With children up to, or even beyond the age of puberty, growth can still be used as the criterion of an adequate protein intake, provided that there is no calorie deficiency. The allowance recommended by previous committees is of the order of 3 g. of protein per kg. per day, of which half should be animal protein. This recommendation, however, is based on recorded intakes in Western countries, and in no way represents the minimum necessary for good growth. Data presented from Central America and Africa showed that on an intake of 2-3 g. of protein per kg. per day, mostly of vegetable origin, growth tends to follow the normal curve, by Western standards, but it is retarded. The children grow, but do not catch up the ground lost in the post-weaning period.

It was agreed that, as an interim proposal, a logical way of formulating the protein needs of children would be by interpolation, "tapering off" from 1·5 g. per kg. at 6 months to 0·5 g. per kg. after puberty. Both these figures are in terms of milk protein. It was emphasized that more must be allowed during adolescence, when the growth-rate is probably increased. There is evidence that the normal 'spurt' may be delayed if the diet is inadequate. Even in the U.S.A. negative nitrogen balances have been recorded in adolescent girls.

In considering the protein requirements of adults we meet the difficulty of the criterion to be applied, since obviously growth cannot be used. Data of two kinds are available—records of actual intakes, and the results of nitrogen balance experiments. In Western countries the recommended allowance is of the order of 1 g. per kg. per day. As *Mayer* pointed out, it is illogical to set the allowance so high, if it has been agreed that 1·5 g. per kg. is enough during the period of most rapid growth. Chittenden, as is well known, maintained that health could be preserved indefinitely on an intake of about 30 g., corresponding to less than 0·5 g. per kg. for an average adult. Rose's experiments lead to

an even lower figure: from his data about 0·35 g. per kg. per day should be enough to provide 'safe' levels of all the essential amino-acids if the protein is of good quality. Records collected by Platt and Scrimshaw in Africa and Central America showed intakes of 1 g. or more per kg. per day. This protein was mainly from vegetable sources, but seemed nevertheless to provide adequate amounts of the amino-acids most likely to be limiting. There is, however, no certainty that these subjects were in optimal health.

Nitrogen balance studies agree with the experiments of Rose in showing that equilibrium can be maintained on intakes of much less than 1 g. per kg. per day. All such observations have, however, the disadvantage that they only cover a short period of time under artificial conditions. *Allison's* work showed that even when there is nitrogen balance, the protein stores or reserves of the body may still be reduced. Who can say that in the long run this is not a disadvantage for an organism subjected to the stresses of everyday life? The discussion thus returned to the problems dealt with in the first session. Since we do not know the significance of the so-called protein stores, and therefore have no criterion of what Terroine called the 'optimum for health', only a compromise conclusion could be reached. This was that, taking the evidence as a whole, an intake of 0·5 g. per kg. per day should be enough for an adult, provided that the protein is of good quality.

The discussion ended with a consideration of the special requirements during pregnancy and lactation. Surveys of the intakes of pregnant women in America show that in fact there is very little increase during pregnancy. This may simply mean that the women were already getting more than enough. However, the amount of new tissue laid down in pregnancy is not in fact very great. By analogy with the growing child, the requirements for protein synthesis in the foetus and adnexa should involve an extra intake of about 10 g. of protein a day over and above that needed for maintenance. The maxim that a pregnant woman should eat for two is therefore a distinct exaggeration.

Lactation, however, does impose a considerable drain on the mother's protein stores. The efficiency of protein conversion in the human being is not known precisely, but by analogy with the cow it may be taken as about 50 per cent. This means that the lactating woman should get an extra allowance of the order of 20 g. of protein a day. Since stress has already been laid on the importance of prolonged and adequate lactation if the infant's requirements are to be met, it is obvious that the protein needs of the lactating mother must be given a high priority.

There were two general conclusions that emerged from this whole discussion: first, that the allowances recommended by previous committees, and based mainly on intakes in Western countries, are at all ages too high, and to reduce them is not only realistic but scientifically sound. Secondly, there is clear evidence that in many parts of the world infants after weaning, young children and nursing mothers do not get enough protein. Some progress was made in defining the needs of these age groups. In the meantime we are faced with the practical problem of making good the deficiency. This was the subject of discussion in the last part of the conference.

References

[1] HOLEMANS, K. (1955). Les carences alimentaires au Kwango. Publication de l'Institut Royal Colonial Belge. *Mémoires de la Section des Sciences Naturelles et Médicales*, **25**, fasc. 3.

[2] HOLEMANS, K. and MARTIN, H. (1954). Étude de l'allaitement maternel et des habitudes alimentaires du sevrage chez les indigènes du Kwango. *Ann. Soc. belge Méd. trop.*, **34**, 915.

[3] HOLEMANS, K., LAMBRECHTS, A. and MARTIN, M. (1954). Étude qualitative et quantitative du lait des femmes indigènes du Kwango (Congo belge). *Rev. méd. Liège*, **9**, 714.

[4] GUNTHER, M. and STANIER, J. E. (1951). The volume and composition of human milk. In Studies of undernutrition, Wuppertal 1946–9. *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 275. H.M. Stationery Office, London.

[5] GUNTHER, M. (1952). Composition of human milk and factors affecting it. *Brit. J. Nutr.*, **6**, 215.

[6] DUFOUR, V. and GOURRY, N. (1934). Les troubles de suralimentation du nourrisson indigène en A.O.F. *Ann. Méd. Pharm. colon.*, **32**, 493.

[7] VENKATACHALAM, P. S. and PATWARDHAN, V. N. (1953). The role of *Ascaris Lumbricoides* in the nutrition of the host; effect of ascariasis on digestion of protein. *Trans. R. Soc. trop. Med. Hyg.*, **47**, 169.

[8] CONFERENCE ON PROTEIN MALNUTRITION (1955). Proceedings of a Conference held in Jamaica, 1953. Ed. J. C. Waterlow. University Press, Cambridge.

[9] DAVIS, T. R. A. and MAYER, J. (1955). Protein requirements. Working paper presented to the Princeton Conference on behalf of FAO.

[10] GELFAND, M. (1954). Kwashiorkor. A description of a case in a breast-fed infant. *S. Afr. med. J.*, **28**, 185.

[11] BROCK, J. F. and AUTRET, M. (1952). Kwashiorkor in Africa. *F.A.O. nutr. Stud.*, No. 8, Rome. *W.H.O. Monograph Series*, No. 8, Geneva.

[12] PLATT, B. S. (1955). Contributions to discussions on 'Human milk versus cow's milk' and 'Amino acid and sulphur content of hair in normal African natives and in kwashiorkor'. *3rd int. Congr. Nutr.*, 1954, *Voeding*, **16**, 147 and 217.

[13] SILVERA, W. D. and JELLIFFE, D. B. (1952). Liver biopsies in Nigerian children. *J. trop. Med. (Hyg.)*, **55**, 73.

[14] WOODRUFF, A. W. (1955). The natural history of anaemia associated with protein malnutrition. *Brit. med. J.*, **1**, 1297.

[15] CASTAÑEDA, G., AGUIRRE, F., GUZMÁN, M. and MÉNDEZ, J. (1955). Estudios nutricionales en un grupo de niños guatemaltecos. I. Del nacimiento a un mes de eded. *Rev. Col. méd., Guatemala*, **6**, 11.

[16] HANSEN, J. D. L. and BROCK, J. F. (1954). Potassium deficiency in the pathogenesis of nutritional oedema in infants. *Lancet*, **2**, 477.

[17] DARROW, D. C. (1947). Advances in treatment of diarrhea in infants. *Tex. Rep. Biol. Med.*, **5**, 29.

[18] DEAN, R. F. A. and SCHWARTZ, R. (1953). The serum chemistry in uncomplicated kwashiorkor. *Brit. J. Nutr.*, **7**, 131.

[19] GOMEZ, F., GALVAN, R. R. and CRAVIOTO, M. J. (1952). Nutritional recovery syndrome; preliminary report. *Pediatrics, Springfield*, **10**, 5.

[20] GABUZDA, G. J., Jr. and DAVIDSON, C. S. (1954). Protein metabolism in patients with cirrhosis of the liver. *Ann. N.Y. Acad. Sci.*, **57**, 776.

[21] SHERLOCK, S., SUMMERSKILL, W. H. J., WHITE, L. P. and PHEAR, E. A. (1954). Portal-systemic encephalopathy. Neurological complications of liver disease. *Lancet*, **2**, 453.

[22] BEST, C. H., HARTROFT, W. S., LUCAS, C. C. and RIDOUT, J. H. (1955). Effects of dietary protein, lipotropic factors, and re-alimentation on total hepatic lipids and their distribution. *Brit. med. J.*, **1**, 1439.

[23] FOOD AND NUTRITION BOARD (1953). Recommended Dietary Allowances, Revised 1953. Publication No. 302. National Academy of Sciences, National Research Council, Washington, D.C.

[24] FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (1950). Report of the Committee on Calorie Requirements. *F.A.O. nutr. Stud.*, No. 5. Rome.

[25] WIDDOWSON, E. M. (1947). A study of individual children's diets. *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 257. H.M. Stationery Office, London.

[26] DEAN, R. F. A. (1953). Plant proteins in child feeding. *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 279. H.M. Stationery Office, London.

[27] CANADIAN COUNCIL ON NUTRITION (1950). A dietary standard for Canada. *Canadian Bulletin on Nutrition*, **2**. King's Printer and Controller of Stationery, Ottawa.

[28] BEAL, V. A. (1953). Nutritional intake of children. *J. Nutr.*, **50**, 223.

[29] MACY, I. G. (1942). *Nutrition and Chemical Growth in Children*. Vol. I. Evaluation. Charles C. Thomas, Springfield, Illinois.

[30] BAPTIST, N. G. and DE MEL, B. V. (1955). Growth and amino-acid intakes of children on a cereal-legume-vegetable diet. *Brit. J., Nutr.*, **9**, 156.

[31] MALCOLM, S. (1953). Nutrition investigations in New Caledonia. *South Pacific Commission, Tech. Paper* No. 50.

[32] HUENEMANN, R. L. and COLLAZOS, C. C. (1954). Nutrition survey of young children in Peru. II. San Nicolas, a cotton hacienda, and Carquin, a fishing village, in the coastal plain. *J. Amer. diet. Ass.*, **30**, 6: 559.

[33] HUENEMANN, R. L. and COLLAZOS, C. C. (1954). Nutrition and care of young children in Peru. III. Yurimaguas, a jungle town. *J. Amer. diet. Ass.*, **30**, 11: 1101.

[34] THOMAS, K. (1910). Über das physiologische Stickstoffminimum. *Arch. Anat. Physiol., Lpz., Physiol. Abt.*, Suppl. Bd., p. 249.

[35] PLATT, B. S. (1954). Infant-feeding practices; breast feeding and the prevention of infant malnutrition. *Proc. Nutr. Soc.*, **13**, 94.

[36] ALBANESE, A. A. (1950). *Protein and Amino Acid Requirements of Mammals*, p. 115. Academic Press, New York.

[37] TECHNICAL COMMISSION OF THE HEALTH COMMITTEE (1936). Report on the physiological bases of nutrition. *Quart. Bull. Hlth Org. L. o. N.*, **5**, 391.

[38] SCRIMSHAW, N. S. and GUZMÁN, M. (1953). The effect of dietary supplementation and the administration of vitamin B_{12} and aureomycin on the growth of school children. National Vitamin Foundation, *Nutrition Symposium Series*, No. 7, and unpublished data.

[39] SOGANDARES, L., DE GALINDO, A. P. and MEJIA, H. P. (1953). Estudios dieteticos de grupos urbanos y rurales de la Republica de El Salvador. *Bol. Ofic. sanit. pan-amer.* Suppl. No. 1. Publicaciones Cientificas del Instituto de Nutricion de Centro America y Panama, p. 27, and unpublished data.

[40] TRÉMOLIÈRES, J. and BOULENGER, J. J. (1950). Contribution à l'étude du phénomène de croissance et de stature en France de 1940 á 1948. *Rec. Inst. nat. Hyg.*, **4**, 117.

[41] TERROINE, E. F. (1956). Les besoins protéiques de l'homme et les conditions de leur satisfaction. *Bull. méd. A.O.F.* In the press.

[42] BRODY, S. (1945). *Bioenergetics and Growth.* Reinhold, N.Y.

[43] PLATT, B. S. (1954). Nitrogen metabolism in malnourished infants and children. In *Report of the Second Inter-African (CCTA) Conference on Nutrition, Gambia*, 1952. p. 153. H.M. Stationery Office, London.

[44] HOLT, L. E. and FALES, H. L. (1921). The food requirement of children: II. Protein requirement. *Amer. J. Dis. Child.*, **22**, 371.

[45] DONELSON, E. G., NELSON, P. M., OHLSON, M. A., PITTMAN, M. S., LEVERTON, R. M., MCKAY, H., KINSMAN, G. M., ARMSTRONG, W. and REYNOLDS, M. S. (1945). Nutritional status of midwestern college women. *J. Amer. diet. Ass.*, **21**, 145.

[46] PITTMAN, M. S. *et al.* (1946). Variations in the basal metabolism of midwestern college women. *J. Amer. diet. Ass.*, **22**, 307.

[47] OHLSON, M. A., BREWER, W. D., CEDERQUIST, D. C., JACKSON, L., BROWN, E. G. and HARRIETT-ROBERTS, P. (1948). Studies of protein requirements of women. *J. Amer. diet. Ass.*, **24**, 744.

[48] MCKAY, H., PATTON, M. B., OHLSON, M. A., PITTMAN, M. S., LEVERTON, R. M., MARSH, A. G., STEARNS, G. and COX, G. (1942). Calcium, phosphorus and nitrogen metabolism of young college women. *J. Nutr.*, **24**, 367.

[49] JOHNSTON, J. A. (1953). *Nutritional Studies in Adolescent Girls and their Relation to Tuberculosis.* Charles C. Thomas, Springfield, Illinois.

[50] WANG, C. C., HODGEN, C. and WING, M. (1936). Metabolism of adolescent girls. II. Fat and protein metabolism. *Amer. J. Dis. Child.*, **51**, 1083.

[51] WIDDOWSON, E. M. and MCCANCE, R. A. (1954). Studies on the nutritive value of bread and on the effect of variations in the extraction rate of flour on the growth of undernourished children. *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 287. H.M. Stationery Office, London.

[52] HEGSTED, D. M., TSONGAS, A. G., ABBOTT, D. B. and STARE, F. J. (1946). Protein requirements of adults. *J. Lab. clin. Med.*, **31**, 261.

[53] FREUDENBERG, E. (1950). In *Lehrbuch der Pädiatrie.* Ed. Fanconi, G. and Wallgren, A. J. Schwabe, Basel.

[54] WATERLOW, J. C. and WEISZ, T. (1956). The fat, protein and nucleic acid content of the liver in malnourished human infants. *J. clin. Invest.*, **35**, 346.

[55] WATERLOW, J. C., BRAS, G. and DEPASS, E. (1957). Further observations on the liver, pancreas and kidney in malnourished infants. 2. The gross composition of the liver. *J. trop. Paediat.* In the press.

[56] BURKE, B. S., HARDING, V. V. and STUART, H. C. (1943). Nutrition studies during pregnancy: relation of the protein content of the mother's diet during pregnancy to birth length, birth weight, and condition of the infant at birth. *J. Pediat.*, **23**, 506.

[57] HUNSCHER, H. A., DONELSON, E., NIMS, B., KENYON, F. and MACY, I. G. (1933). Metabolism of women during the reproductive cycle. V. Nitrogen utilization. *J. biol. Chem.*, **99**, 507.

[58] DARBY, W. J., MCGANITY, W. J., MARTIN, M. P., BRIDGFORTH, E., DENSEN, P. M., KASER, M. M., OGLE, P. J., NEWBILL, J. A., STOCKELL, A., FERGUSON, M. E., TOUSTER, O., MCCLELLAN, G. S., WILLIAMS, C. and CANNON, R. O. (1953). The Vanderbilt co-operative study of maternal and infant nutrition. IV. Dietary, laboratory and physical findings in 2,129 delivered pregnancies. *J. Nutr.*, **51**, 565.

[59] GARRY, R. C. and WOOD, H. O. (1946). Dietary requirements in human pregnancy and lactation; review of recent work. *Nutr. Abstr. Rev.*, **15**, 591.

[60] GARRY, R. C. and STIVEN, D. (1936). Review of recent work on dietary requirements in pregnancy and lactation, with attempt to assess human requirements. *Nutr. Abstr. Rev.*, **5**, 855.
[61] SCRIMSHAW, N. S., THOMASON, M. J., BAYS, R. P. and HAWLEY, E. E. (1949). Nutrition of women during normal and abnormal pregnancy in Panama and the Canal Zone. *Fed. Proc.*, **8**, 396.
[62] GREULICH, W. W. Personal communication.
[63] GOETTSCH, M. (1949). Minimal protein requirement of the rat for reproduction and lactation. *Arch. Biochem.*, **21**, 289.
[64] LEAGUE OF NATIONS (1936). The Problem of Nutrition. Vol. II. Report on the physiological bases of nutrition. League of Nations, Geneva.
[65] DARBY, W. J., DENSEN, P. M., CANNON, R. O., BRIDGFORTH, E., MARTIN, M. P., KASER, M. M., PETERSON, C., CHRISTIE, A., FRYE, W. W., JUSTUS, K., MCCLELLAN, G. S., WILLIAMS, C., OGLE, P. J., HAHN, P. F., SHEPPARD, C. W., CAROTHERS, E. L. and NEWBILL, J. A. (1953). The Vanderbilt co-operative study of maternal and infant nutrition. I. Background. II. Methods. III. Description of the sample and data. *J. Nutr.*, **51**, 539.
[66] SHUKERS, C. F., MACY, I. G., DONELSON, E., NIMS, B. and HUNSCHER, H. A. (1931). Sources of nutrient chosen by women during pregnancy, lactation and reproductive rest. *J. Amer. diet. Ass.*, **7**, 235.
[67] SHUKERS, C. F., MACY, I. G., DONELSON, E., NIMS, B. and HUNSCHER, H. A. (1931). Food intake in pregnancy, lactation and reproductive rest in the human mother. *J. Nutr.*, **4**, 399.
[68] SHUKERS, C. F., MACY, I. G., NIMS, B., DONELSON, E. and HUNSCHER, H. A. (1932). Quantitative study of dietary of the human mother with respect to nutrients secreted into breast milk. *J. Nutr.*, **5**, 127.

III: PRACTICAL MEASURES TO INCREASE PROTEIN INTAKES

Utilization of Local Resources

INTRODUCTION

BURGESS: In protein malnutrition we are confronted with a very difficult public health problem—perhaps the most important in the whole field of public health in many countries throughout the world today. As direct information accumulates from records of hospital admissions, and indirect statistical evidence from records of the age at death in young children, the great significance of the disease becomes more and more apparent.

In the prevention of protein malnutrition obviously one of the most important measures is the development of more good protein-rich foods. The FAO WHO Joint Expert Committee on Nutrition, at its Fourth Session in Geneva in November, 1954, made a statement on this which I would like to quote:[1]

". . . Meanwhile it should be emphasized that in many parts of the world supplementary foods for the breast-fed infant and foods for weaned infants and young children are seriously lacking. Although milk is the usual infant food in most developed and many under-developed countries, there is need for the introduction of foods which utilize other good sources of protein for the mixed feeding of infants and for improving the diets of children. Some of these will also be of value for bettering the nutrition of pregnant and nursing women. These measures usually need to be developed on a regional basis, and will depend on the availability of local products and the nature of local food habits. . . ."

The question that confronts us now is what can we do to help achieve a satisfactory solution. The previous meeting in this series in Jamaica established contacts between workers engaged in this problem, and these contacts have had a most important influence on the progress of the work in different laboratories. WHO have followed this up by assisting in the exchange of workers between India, Africa and Latin America and we have made some small provision for continuing this next year. As a result, there are workers in different parts of the world who know each other's problems and how it is proposed to try and solve them, and who are also aware of the techniques for investigation and know the detailed plans which are being developed. In addition, we have been able to make some small financial grants to help these laboratories to pursue their efforts to find such foods, but the sum of money available is small in relation to the size of the problem. This meeting offers us another opportunity for an exchange of views; we hope that, perhaps, out of these discussions may emerge some principles which will serve as guides in the development of these programmes.

A matter which deserves a very great deal of consideration is the preliminary testing of these foodstuffs. This, I think, is an extremely important matter, for the introduction on a wide scale of any public health measure which is harmful, or even which is not effective, may do damage that is difficult to retrieve. If such a mistake is made in an educated community, the harm done may not be very great, for the whole matter can be explained and understood. On the other hand, the introduction of an unsatisfactory new foodstuff to an uneducated community may do irreparable damage, for its failure often cannot be explained in such a way that it can be understood, and thereafter the fault is regarded as

something inherent in the foodstuff itself. This may prevent any further development along these lines. The preliminary testing is a matter of very considerable importance and we would like to hear views on this matter.

Another matter in which we would like help is also brought out in this expert committee report.[1] The committee drew attention to the fact that the technical and other resources required for these studies frequently do not exist in the countries in which there is the greatest need for foods for the infant and young child. The total resources are small and we would like consideration to be given as to how they can best be utilized. I would like to put these two main problems before the group.

FREMONT-SMITH: I think one of the most valuable things that WHO has done in this programme is the exchange of workers. I can't imagine any method of spending limited resources more likely to further the purposes with which WHO and FAO and all of us are concerned than this particular provision.

AUTRET: In tackling protein malnutrition as a public health problem, it is a first principle of FAO that we should try to develop milk production wherever feasible. The next approach is how to use for feeding children various protein-rich foods now used for animal feeding only. Of one million tons of fishmeal now being produced in the world, 30 to 40 per cent, by the most conservative estimate, could be used for feeding children. In Africa alone I know that 500,000 tons of peanut meal are available, which might well be turned into an edible protein food; there are also large quantities of soya flour.

The problem is how to utilize these commodities in feeding children, and to do it without competing with animal feeding. Part will be fed to animals in the well-developed countries; part will remain with the children in the less-developed countries, because the milk production of cows fed peanut, fishmeal, etc., will still not be enough to meet needs and the price will be too much for the poor. It is among such people, with little or no money, that protein malnutrition occurs.

We would like at this meeting to find out what is known and what is not known, so that we can proceed with the research needed. From what is known, what trials are required before the commodity can be used as a food for children? What processing is needed? There will be difficulties then to be faced in the field of economics, food technology and education, but these can be dealt with elsewhere. The problem we must consider now is one for the paediatrician—a technical and biochemical problem, not one of agricultural policy.

GYORGY: One of the rare and truly gratifying experiences in recent medical scientific progress has been the rapid development in our knowledge of kwashiorkor as one of the pressing nutritional and public health problems of the world, particularly in the so-called under-developed countries. One has really to admire the speed with which the background of this dietary condition and the underlying problems were unravelled. It is only four years ago that the report of Brock and Autret[2] was published, and since 1951 there have been several other scientific conferences and major publications.[3, 4, 5]

We have, I think, reached a stage which is absolutely essential for any practical action programme. It is always naïve to think that if experts come together they can solve any problem, although wartime experience led the layman to believe that that could be done. In the case of kwashiorkor, however, we have really progressed to a point where a practical approach is feasible. But we cannot now relax and feel we have done our job as scientists, for the purpose of an action programme is twofold: purely practical, utilitarian,

perhaps, as a first step, even if it is not a complete solution; and secondly, still further research into the causes of protein malnutrition, and into the means of finding a better solution.

The practical approach, again, is twofold: treatment and prevention of protein malnutrition. Treatment has been discussed in detail at the last conference in Jamaica, and to some extent at the earlier sessions of this meeting. Here we are concerned with prevention. Dr. Burgess has already mentioned that the greatest need in prevention is to find supplementary food preparations containing available protein, with sufficiently high biological value, which are acceptable to the country in question, and of course cheap. Dr. Autret rightly said that we are not concerned at the moment with agricultural policy, but with the biochemical and medical aspects of an action programme. As a paediatrician I must emphasize that if any new supplementary food for young infants is introduced into a country and has not been used before, it must be looked upon as a drug. As such it needs prolonged and thorough testing, and this is an aspect that we must consider very carefully.

KING: From these introductory remarks it seems to me that the action programme has to be considered under two main heads: (1) the nature and potential value of foodstuffs that may serve as sources of supplementary protein. This again may be divided into two parts:

(*a*) Foods that may be grown in the district or the village, and for which any necessary processing may be done in the home. Here conditions will naturally vary from region to region.

(*b*) Sources of protein that have to be developed and processed on the industrial scale. Here problems of economics and distribution may become extremely important.

(2) The second main topic is this: supposing that sources of supplementary protein are available, how are we to test their effectiveness and be sure of their safety, before they are fed to infants and pregnant and nursing women? This is the point particularly emphasized by Dr. Gyorgy.

I suggest that we might take the topics in that order. We are, of course, mainly concerned with guiding principles.

REGIONAL CONSIDERATIONS

GOPALAN: The general premise seems to be that protein malnutrition in under-developed countries is directly and solely the result of the primary dietary inadequacy of protein, and that we should be able to evolve a uniform blanket solution applicable to all regions.

We know that protein malnutrition is a global problem, but it has its regional peculiarities. There may be a good deal of unanimity in the clinical picture, but I believe that the aetiological factors in different regions are different. I feel that a greater contribution to the prevention of protein malnutrition in certain regions of the world may lie, not in supplying supplements, but just in eradication of malaria, the improvement of poor home economic conditions, etc. Therefore the first thing we should particularly emphasize here is that there are these factors varying from region to region, which are responsible for the precipitation of protein malnutrition, and which must be taken into account in planning any action. There is no blanket solution which will apply to all parts of the world. We must therefore speak of plans for different regions, and the formulation of such plans should be preceded by carefully conducted surveys. These should not

be confined to assessing the incidence of protein malnutrition in that region, but should also include contributory factors which may well be anthropological, sociological, epidemiological, etc. Finally, the survey should take note of the available foods in the region.

I think that would be the correct approach. The problem of protein malnutrition is not going to be solved by just processing some foods, and handing them out to the people. I feel that the battle has to be fought in the different regions, with a different solution for each.

DEAN: The point made by Dr. Gopalan is one that we always come up against in discussions of this sort. We have to know where to start. I think we already know enough about the general principles of the supplementation of foods and the use of one food in association with another. What we are faced with is to a very large extent a technological problem to find the way to use these foods in children's diets.

AUTRET: I agree in many respects with Dr. Gopalan. Our discussion should proceed from the general to the particular, and we should start by a review of the protein-rich foods available in the world or in various regions. We should also know what is the staple food in each region and study how the protein-rich foods available might supplement the basic diet. Therefore we must also pay attention to the mother's diet, and to the development of protein-rich supplements which the mother can utilize much better than the child.

Up to the age of 6–8 months the child must be fed breast milk or cow's milk. Thereafter he must receive some additional food, including food rich in protein. Part of this additional food will in many cases be the basic traditional diet of the family. Therefore, when we consider supplementing the child's diet with some good-quality protein, it is not simply a matter of gross quantity about which one can generalize. We have to consider what he is getting from the family pot. This may vary with many things, including the economic status of the family.

In FAO we are now reviewing the cheap, protein-rich foods which are available or might be available. Much of this information is summarized in a recent report.[6] We are not considering meat or eggs, which are expensive and on which we have adequate information, but rather those foods which have been little used or not used at all for feeding children, and to which some objection has been raised, such as fish flour. I will tell you more about that later.

We believe that no food would be accepted by the mother for the child in an under-developed area if she herself declined it. At least it would be easier to introduce a food which the mother knows and likes than a food she does not know. Therefore, when we worked with fish flour in Africa, for example, we tried to find a tested product which the mother would accept. These are some of the general principles which guide us.

CRUICKSHANK: I suggest that at this stage we look more carefully into natural foods, and refer back to our old baseline suggested by Dr. Terroine. We should examine some of these products now with a view to their amino-acid content, and therefore their suitability as a basic food, and what possible mixtures of these natural foods may be introduced into the diet of the community without, in the first instance—and I emphasize "in the first instance"—considering the problem of "processing", which introduces the additional factor of cost.

TERROINE: I entirely agree with Dr. Cruickshank's suggestion. As I stressed in the report that I presented to the conference,[7] the essential starting point for all our studies should be to find out what exactly are the various resources in a

given region. If we want to make recommendations that may be of some value, we cannot give identical advice for all the regions of the world.

ANALYSIS AND STANDARDIZATION

TERROINE (continued): In this connection, I would like to mention four general points. The first is that we should tell all the regions of the world that they should continue as hitherto to carry out analyses, both chemical and biological, of the basic foods they use. An important point which has been raised by Dr. Autret is that we are here talking about food supplements, and if we are to decide what supplement to give, we must first of all ascertain what is lacking and what has to be supplemented in the basic food.

ROSE: I would like to support that strongly. The only logical way to select the best product for supplementary feeding is to accumulate information on the amino-acid distribution in the diets actually consumed. I believe that a great deal of information is already available, and it would not be a very long-term programme to do this in many regions.

SÉNÉCAL: I agree that it is essential to establish the amino-acid composition and to measure the biological value of the foods we propose to use as supplements. The trouble is that analyses have been made in different places with entirely different results. This has happened, for instance, with peanuts. It may be due partly to differences in the nuts themselves, but partly also to differences in techniques. It might be useful, therefore, to centralize all this research, both for chemical analysis and even more for biological testing.

TERROINE: I fully agree with Dr. Sénécal. I have myself proposed the creation of a central office to which would be entrusted the task of analysis by agreed standard methods. The office I proposed would have been on the pattern of the statistical office that is to be set up by UNESCO in the very near future.

I don't know whether it would be possible to implement this proposal, which, I should add, would not be a very costly one, but in the meantime I think we could at least concentrate on achieving some kind of international standardization of our methods. Very good work has been done in this field by Dr. Allison in particular, but it is possible that the differences in results mentioned by Dr. Sénécal would be entirely eliminated if we were to reach an agreement on the methods of evaluation. It happens very often that results obtained by bacteriological methods are compared with results obtained by chemical methods; or the same analytical methods may be used, but hydrolysis carried out by different procedures.

It would be very useful if we could set up a small group of biochemists, who could agree on standard methods for use in this field. I am trying at the moment to do it in France, but I think there is even greater need to do it for the whole world.

SCRIMSHAW: I am sure that Dr. Sénécal and Dr. Terroine mean to distinguish between a central laboratory for the final checking of regionally developed products, and the use of a central laboratory to assist the regions in the development of supplementary foods. It would be impractical to send each new foodstuff or mixture several thousand miles for chemical analysis or for animal trials. Regional development—and it must be regional—requires regional laboratory facilities. On the other hand, the collection and distribution of information on the amino-acid analyses that are being carried out in the different laboratories around the world and the final checking of regionally developed products would be an extremely useful and important contribution.

DEAN: We have been trying to standardize the biochemical methods used in the study of kwashiorkor. When we recently summarized the literature we found an extraordinary confusion. The next stage in the same programme seems to be standardization of the analysis of food materials. Different laboratories certainly get extraordinarily different results. I think that some central body would do such things better, or would be a very useful check on the work done regionally. The right approach is by centralization, which obviates the duplication and the doubtful circumstances of the various analyses in a large number of stations.

TERROINE: The next point I want to make is that in our methods of determination we must make use not only of chemical analysis but also of biological analysis. The chemical analysis in itself is not sufficient. Admittedly it gives us important information about the content of amino-acids, but that alone is not enough, because we know that the availability and the rate of liberation of amino-acids play a very important part. One can easily imagine that two proteins might have an identical overall amino-acid composition, but might differ in the arrangement of the amino-acids within the molecule. As a result, their biological values might differ considerably. Lysine is a striking example. If a product is heated to 150° C. for 3 hours, the lysine content is hardly altered, but this lysine can no longer be liberated by hydrolysis in the intestinal tract. Therefore the biological value of the product is considerably altered, but this cannot be detected by chemical analysis.

EXAMINATION AND EVALUATION

TERROINE (continued): My third point is that we must continue our examination of animal and vegetable proteins, to find out which of them can be used most successfully as a supplement. Here, of course, there are differences in approach, according to whether the foodstuff we are interested in is to be used as a supplementary source or as the sole source of protein. Very often a protein which is of extremely low value by itself can constitute a perfectly good supplement. For instance, in cereals there is a deficiency in lysine and the sulphur amino-acids which can be corrected by the use of gelatin; this by itself is totally unable to supply the nitrogen requirements, but is rich in lysine. My main point, therefore, is that we must first ascertain what is lacking, and that of course would help us to determine what should be added.

We can reach very satisfactory results with mixtures of vegetable proteins alone, as has been pointed out by Drs. Scrimshaw, Dean, Gomez and others. It is well known, for instance, that a mixture of rice and wheat can increase the growth rate by about 100 per cent, compared with rice alone.

The fourth point I want to mention concerns the method of evaluation. This is, indeed, a most delicate problem. We are never dealing with proteins in a pure state, but, rather, with protein foods, and these protein foods naturally also contain substances such as minerals and vitamins. How are we to determine the protein efficiency of a given food? The best method—and it has been applied by many people engaged in cattle rearing and in experiments with animals—is to compare the nitrogen retention with that obtained on an ideal protein. I think this is an excellent method, provided that in these comparative trials the two diets are adjusted to provide identical intakes of energy, vitamins and minerals, so that there is only one variable, the quality of the protein. Only then, when we have determined all these points, can we turn to technological and economic considerations.

Measures Adopted in Various Regions

METHODS OF APPROACH

SCRIMSHAW: I should like to indicate the general philosophy and course of action which we are trying to follow in Central America—not that this is going to be specific to our region. I am sure that there is a very large measure of agreement about this process.

We recognize at the start that there are a variety of social, economic and cultural factors, as well as disease factors, which need attention, but there is a basic problem of protein shortage.

In the first place, we are interested in increasing the production and availability of animal products by all means that are practical, and we are working very actively toward that end in co-operation with the agricultural agencies. But at the same time it is obvious that even with a manifold improvement in animal production, there will be a shortage of adequate protein of reasonable quality, and that part of the solution must come through the improved use of vegetable proteins. This can come about in a number of ways, each of which must be explored and developed: the use of a greater variety of vegetable proteins in the ordinary diet for their complementary effect, the enrichment of a common product such as tortilla with certain specific vegetable proteins, and the possibility of developing cereal and other food mixtures which can be given to the child as a supplement after weaning.

From our experience to date, we are convinced that it is practical to develop a combination of plant proteins, locally available, which are suitable for this purpose, and we think that it is something that has to be done regionally. We don't think in terms of developing a product in Central America necessarily suitable for South America, and certainly not for Africa and other parts of the world.

In developing such a product, we feel that we have a real responsibility to be extremely careful. We need to know the chemical nature and nutrient composition of the vegetable products and other food products which we are using, and then to carry out careful animal testing. We use baby chicks for the preliminary screening of plant products and testing of mixtures. In the final trials we are using the rat and the baby pig. Not until the tests have been completed in those animals do we feel justified in giving the product in therapeutic amounts to hospitalized children, and then only under careful supervision.

We may introduce a small amount into diets earlier than that, just to test acceptability, but as far as the systematic testing in children goes, we feel that it must be done first in a group of hospitalized patients, then extended to a larger group of patients in institutions, and finally tested in field trials.

We must also be extremely careful about the practicality of the technological procedures required, and the economics of whatever mixture we are testing. It is axiomatic that we have to consider, from a very early stage, the anthropological, cultural, social, economic and educational factors which are going to be involved in the use of any new product. There is no point in working on a product without considering from the beginning how we are going to get people to use it.

Finally, when we come to the problem of trying to appraise the value of a product for child feeding, we come to these obvious requirements: (1) that the materials be locally available; (2) that they be cheap; (3) the product be easy to transport and to store; (4) that it be safe; (5) that it be acceptable—and (6)

that it be effective. Of course, all the other characteristics are meaningless unless it is effective. But its biological effectiveness is also meaningless unless these other criteria are fulfilled.

GOPALAN: I think that our approach in India has been somewhat along the lines suggested by Professor Terroine. We first surveyed the problem to find out its extent, and in the course of this survey we collected data for the actual protein intakes of children between 6 months and 5 years old. We have tried, as Dr. Elvehjem and Dr. Rose have advocated, to review these intakes on an amino-acid basis, so that we could assess the deficiency of protein from a qualitative as well as a quantitative point of view. Table 38 shows the amino-acid intakes compared with the minimal requirements as summarized by Dr. Holt in the table he showed us in the first session (Table 5, column 9, p. 22). We see that the dietary intakes of some amino-acids are low in these children; so that in the first place we know exactly what the problem is.

TABLE 38

Comparison of amino-acid intakes of poor South Indian children in various age groups with ideal requirements

Intake (mg. per kg. per day)	Age (years)				Overall minimal requirements*
	Up to 1	1–2	2–3	3–5	
Arginine	69	103	132	155	67
Histidine	29	36	35	36	26
Isoleucine	102	104	98	104	90
Leucine	143	142	132	139	210
Lysine	94	97	87	90	105
Methionine ..	33	35	34	37	31
Phenylalanine ..	82	94	91	95	90
Threonine	63	62	58	62	60
Tryptophan ..	26	20	18	18	30
Valine	123	108	99	102	120

* According to data summarized by Holt in Table 5, column 9, p. 22.

Having got that baseline information, our next step was to survey the potentialities in the region from the point of view of food production. We have examined the available local foods which could be used by the people and which, according to agricultural experts, could be produced on a sufficiently large scale, and have investigated their essential amino-acid composition. The results obtained so far are shown in Figure 20.

We now find from some of our experiments that in addition to cereals and pulses it would be possible to use also various green leafy vegetables which in the past we used to ignore from the point of view of protein nutrition. Therefore, the important and encouraging thing we find is that it should be possible, by judicious combination of products available locally, to arrive at mixtures which could be satisfactorily used in these children.

We do not depend only on estimation of the essential amino-acid composition to decide the suitability of protein foods, because as Professor Terroine has very rightly pointed out, we do find that there is considerable divergence between the biological values of these proteins as determined by animal experiments,

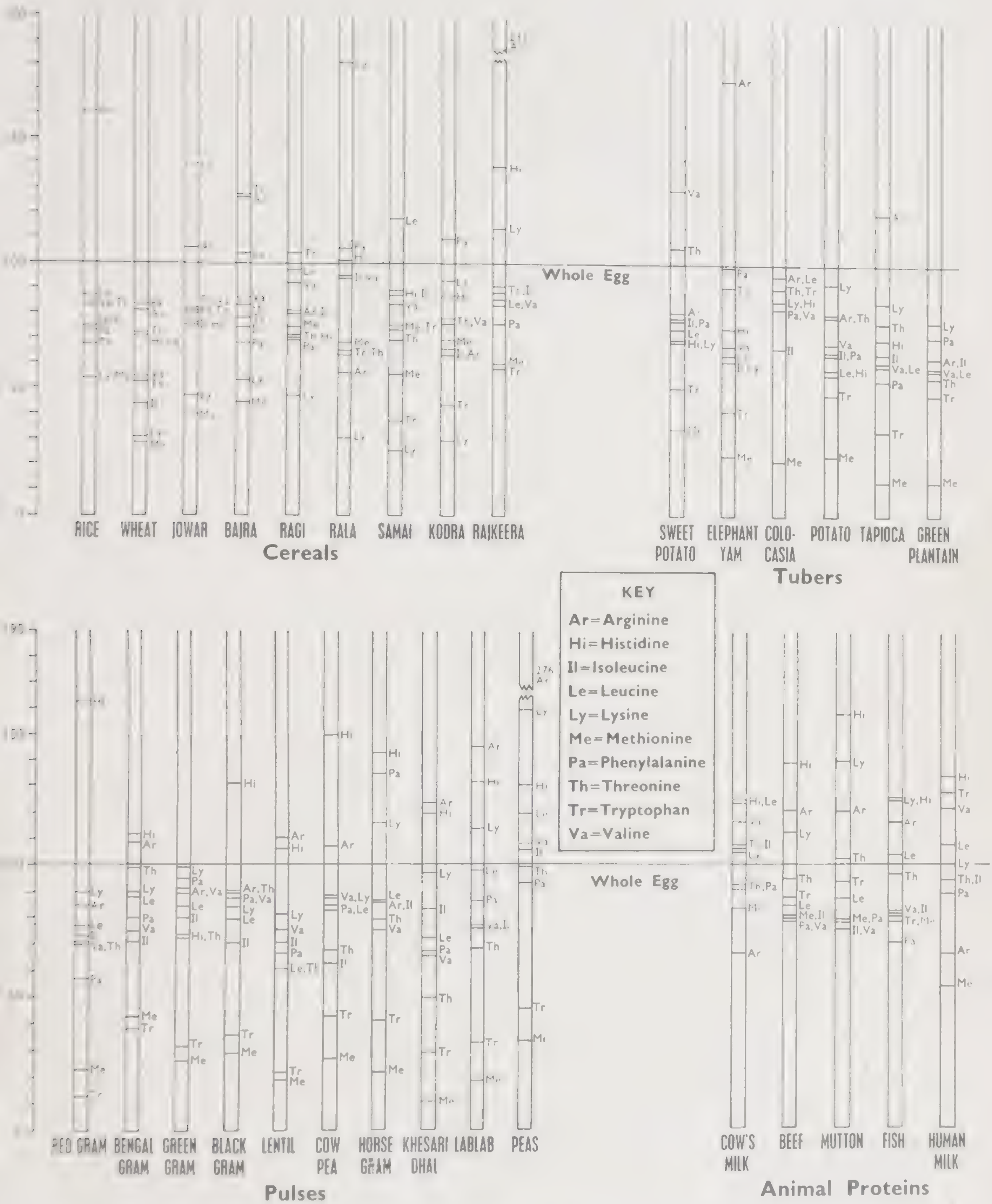

FIG. 20. Essential amino-acid content of the proteins of some Indian foods in relation to that of whole-egg protein. (See Balasubramanian *et al.*,[8] Vijayaraghavan and Srinivasan,[9] Phansalkar and Ramachandran.[10])

and the biological value as calculated by the chemical score technique. We find this particularly in the case of vegetable proteins, so we have depended on animal experiments to assess the suitability of these combinations. We try them not only on normal animals but also on ones which have been protein-depleted for some time. The question is not only to find out which two products can be combined, but in what proportion they should be combined.

Another very important way, in our opinion, of investigating the suitability of these products has been to try them in cases of kwashiorkor, although I know this is controversial. It seems to me an economic, time-saving, and straightforward method of choosing the right combinations of food to be used for the prevention of the disease. It may be argued by some of the participants here that to assess the suitability of the product by the response which it evokes in a disease would be wrong when you are considering it from the preventive standpoint. We feel that that objection is not entirely valid, because, for one thing, the scope for demonstrating differences in the nutritive value of proteins is very much greater when you are dealing with these depleted subjects than with apparently normal children. Secondly, there is the argument that the great majority of the children who stand in need of these protein supplements are already malnourished. They may not be actually suffering from kwashiorkor, but they may be in the stage of "pre-kwashiorkor" or one of the various grades of protein malnutrition; so we think that this approach is a valid one, and in our opinion it has yielded apparently valuable results.

We have tried to assess the preventive value of combinations containing Bengal gram and various cereals. These studies are now in progress. We have three field stations in different localities where we intend feeding these products to children, and comparing the results with those from a control group of children who would be getting the UNICEF skimmed milk.

When it comes to the processing of foodstuffs, in the conditions that prevail in India it seems preferable not to think in terms of over-centralized and very elaborate processing. The dietary habits are very variable, and the type of processing and the type of food which would be useful in one region would be found unacceptable in another. We are therefore thinking of something which could be carried out on a regional level. The other great advantage of such an approach, it seems to me, is the point emphasized by Dr. Dean, that some of these elaborate processes may be self-defeating in the sense that they put up the cost of the product.

PLATT: I think it is an obvious point, but worth mentioning here, that the questions of control, if they begin to be elaborate, are outside practical possibilities. For example, in certain under-developed countries methods such as enrichment have been shelved because of the inability to set up, finance and staff the necessary control measures.

SCRIMSHAW: I should like to quote a simple, specific example to show how similar our approach is to that of Dr. Gopalan. In Central America they are planning the large-scale production of a tortilla flour, of a type now being produced in Mexico and on a small scale in Guatemala. In El Salvador there are over 800,000 people fed by their employers, who give them tortilla, a diet which is deficient in vitamin A and in good quality protein. We were asked what could be done with this tortilla flour to make it of better nutritional value.

We investigated the matter by theoretical calculations and by biological trials in chicks and rats. It turns out that the addition of 5 per cent sesame oil meal and 0·5 per cent of a leaf meal made either from ramie or Kikuyu grass, does

considerably improve the performance of depleted animals given this tortilla flour. Moreover, the ingredients are cheaper than the tortilla flour itself. After acceptability tests, which were carried out in both Guatemala and El Salvador, the manufacturer in El Salvador has agreed that the product, from the beginning, will be put on the market with these additions. The small proportion of leaf meal makes a marked difference in the vitamin A activity of the product.

AUTRET: The problem of improving child nutrition, as Dr. Gopalan said, lies in the home; this we have to study at the village level. There is no doubt about that. Therefore we have accepted from the very beginning as our first objective the improvement of village food production and nutritional education. But now we are looking to see if large supplies of cheap protein-rich food, which are available at the world level, can be introduced into the child's diet. All these supplies, which are mainly pulse presscakes—by-products of the oil industry—need some processing first. Here we are introducing a new element, because the quality of the original material, which already varied in itself, will vary much more after processing, and therefore we have to reach some agreement about the method of processing and of testing in order always to achieve the same product under similar conditions.

DARBY: It seems to me that we are really considering two problems here, which get confused because they have different requirements. One is the very important problem, championed by Dr. Gopalan, that I would term dietary betterment through the introduction of variety into diets composed of one cereal or one staple food. The other is the development of concentrates, as the animal-husbandry men call them.

When we consider amino-acid analysis, if we are dealing with food, there are thousands of things that can be analysed. If we are dealing with concentrates, it would be much easier for such analyses to be done or checked in a standard laboratory. I think it would help us to make progress if we could separate these two problems.

DEAN: There is even a preliminary problem that has hardly been mentioned at all; namely, that there is not enough total protein for the children. It wasn't until I worked in the countries which are under-developed that I realized the extent of the sheer absence of protein.

The point from which we start is that we must do everything we can to increase the amount of protein available in the village, and to make it available to children in a form in which they can use it to the best advantage. The lack of protein is fundamental. I may be considered to be in the Dark Ages because I am not so much concerned with the amino-acids, but if children are trying to live on 5 or 10 g. of protein a day, the quality of that protein is of secondary importance.

SCRIMSHAW: But this varies with the region. In some regions the problem is more one of quality than of quantity, and in others it is both.

KING: I agree. For example, conditions are very different even within Central America. In Panama rice is the dominant cereal, but in Guatemala the food supply is chiefly corn and beans. There is a very different problem in improving the dietary in those two areas.

IMPROVEMENTS TO THE PRESENT DIET

KING: Dr. Gopalan, what are the kinds of agricultural produce which you think, in India, would lead to a betterment of the people's own protein sources at the home or village level?

GOPALAN: At the moment we are concentrating in India on two aspects. We are trying to promote fish culture in rice fields and to increase the fishing industry around the coasts. But here we are running up against the problem of transport. As to the vegetable sources of protein, as I explained previously, we are concentrating on pulses, and lately also we have begun to explore green leafy foods. When the animal experiments, the clinical work and the pilot field trials are completed, if we find a particular combination of these vegetable foods which is suitable, it is that combination which will be processed, if necessary on a large scale. That is why I think there is no fundamental distinction between the betterment of the diet, to use Dr. Darby's term, and the supply of concentrated foods.

KING: It would be useful to have similar information from other areas.

DEAN: Dr. Gopalan has made an extremely good point when he spoke of continuing the programme from the raw material to the processed material. We, too, think of the two as part of the same programme.

In Uganda there is the possibility of increasing pulses, which are familiar foods and are well liked. The increase is very largely a matter of ensuring good methods of storage. The other materials, which are important, are peanuts (groundnuts) and sunflower.

KING: You have household or village methods of handling all three of those —pulses, peanuts, and sunflower?

DEAN: Yes. The household method of handling sunflower is extremely time-consuming, but it is being used.

SÉNÉCAL: In Dakar we produce mostly millet; over 20 varieties have been analysed in our part of Africa by the special office in charge of that work, so that we are now well acquainted with the chemical composition, as far as amino-acids are concerned, of all existing varieties of millet.

As far as supplementary foods are concerned, we have tried, first, to use fish flour manufactured from locally available fish, and also fish flour that was sent to us through FAO from South Africa. Recently we have also been making use of peanut flour; peanuts, as you know, grow in Senegal, but have been used until now almost exclusively for cattle. We are now using this peanut flour as a supplementary food. More recently still we have been experimenting with a food sent to us by a dairy company, which is a mixture of various kinds of cereals and fish flour.

DE MAEYER: There are three things I would like to mention: fish, cattle and agriculture. The government of the Belgian Congo is increasing fish production by the creation of fishponds. In the regions near the coast the fish obtained from the ocean are specially frozen and then sent for local consumption to the interior of the country. Special plans are also being set up to convert the fish into fish flour, especially in the interior. Then in the south-eastern part of the country we are trying to increase the number of cattle. As for agriculture, more and more efforts are being made to shift a part of the production from manioc (cassava) to peanuts. There is a large amount of peanut and cotton-seed cake available now. Tests are at the moment being made in Brussels on the amino-acid content of these products.

SCRIMSHAW: In Central America the total intake of protein in the adult population is frequently well above recommended allowances. I have already quoted some figures which show this. Our problem is not to increase the total amount, but to introduce more variety into the diet, so that the net result will be of better biological value. This involves emphasis on variety, and it involves an educational programme.

The pre-school child, as I have already shown, actually does suffer from an acute shortage of protein. This is not only because protein foods are scarce, but also results from ignorance and the lack of satisfactory feeding practices. Beans and meat, even when they are available, are likely to be withheld from the weaned infant. We are counting on education to be of great help over this, and we are also counting on producing a large volume of a cereal product which can be cheaply available for pre-school children.

BENGOA: In Venezuela only 30 per cent of the population can be considered to be consumers with capacity to buy. The remainder rely on the products of their own cultivation. Eighty per cent of the calories and 70 per cent of the protein come from these two foods. We have not carried out experiments on the supplementation of proteins, but soya was a failure when it was introduced.

SCRIMSHAW: In Central America a great deal can be done to increase animal production: in Guatemala, for example, 80 per cent of the land is not suitable for food crops. About half of this, however, is suitable for animal production, primarily through use for forage crops. In co-operation particularly with Dr. Robert Squibb in Guatemala and with personnel in other agricultural stations, we are concerned with helping to improve the local forages, and with the search for ones that are better adapted to the region. We are also helping in the production of local concentrates suitable for animal feeding, and in the introduction of mineral mixtures to correct the almost universal dietary deficiencies of essential minerals.

In working to improve plant proteins, we have given considerable attention to the possibilities for the genetic improvement of corn. In Guatemala corn provides 70 per cent of the protein in many rural areas, in El Salvador 60 per cent, and only slightly smaller amounts in the other Central American countries. Previous studies have indicated that a given amino-acid may vary as much as 100 per cent from one local variety of corn to another.[11, 12] In co-operation with the Rockefeller Foundation, we are testing 25 varieties, grown in eight countries, and in several locations in each country, in the hope that we will be able to select varieties with greatly increased yield and improved nutritive value. Somewhat similar work is also under way with beans.

PLATT: I heard recently of a successful attempt to breed cassava by crossing a variety which has 10 per cent protein with the one that has only 1 or 2 per cent. Considering the extent to which the cassava is spread throughout the world, this means an enormous jump in protein intake. I think we could concern ourselves with matters like that, which might be far more important than the processing of industrial waste products.

HANSEN: I would like to add to Dr. Scrimshaw's point that proteins from different varieties of the same plant may be different in composition and food value. Not only does that apply to different varieties, but also the same variety of plant may produce proteins differing in composition when grown on different soils. Our unit in Pretoria has found that maize grown in one part of the Transvaal is quite different in nutritional value from the same variety grown in the Free State, so soil composition enters into it.

SCRIMSHAW: These differences are highly significant, both the genetic and the environmental, and it is a complicated problem to sort out the two.

CATRON: A number of different plant proteins have been mentioned, but from the work that has been done in the rat, the pig and the chick, soya-bean protein, when properly processed, is without question the superior protein.

Wherever that can be adapted, it should be grown. I don't know why it failed in Venezuela, but that should be looked into.

SCRIMSHAW: The attempt to introduce soya bean into under-developed countries has usually been made without careful plans for its processing and use, without instructions as to how to incorporate it in animal feeds, etc.

CATRON: That is unfortunate, because, once it fails, it is hard to revive it again.

FREMONT-SMITH: This emphasizes the importance of not introducing something into a country without taking great pains to see that it can be used and is acceptable, otherwise it may be prematurely rejected.

CATRON: From the point of view of animal husbandry and agriculture, two aims, it seems to me, need to be foremost in our minds—the production of milk where milk can be produced, and secondly, the production of soya beans as the source of protein where soya beans can be produced. When these fail, then other possibilities, such as fish and other plant proteins, have to be tried.

SCRIMSHAW: I should like to add that there are some very exciting possibilities for forages in tropical areas, where previously there has been no satisfactory forage grown that was adapted to the temperature and soil conditions. Several have been found which should make possible a great improvement in animal husbandry in the tropics.[13, 14]

KING: I was also very much impressed, in each of the Central American countries, where the introduction of silage had completely changed the whole pattern and outlook for local milk and livestock production.

Sources and Relative Values of Protein-rich Supplements

AVAILABLE SOURCES

AUTRET: Most of the points have been discussed many times at FAO general conferences, technical committees and meetings of the joint FAO WHO Expert Committee on Nutrition. For instance, I have already said that it is a first principle of FAO policy to develop milk production wherever possible. We all agree on that. The problem that we need to consider is the following:

There exist at present sources of cheap protein-foods which could be satisfactorily used as human food, at least as a supplement to the local basic food, and which have not, so far, been used. What are these various food sources? What are their present or potential availabilities? What do we know of their nutritive value, of their digestibility, and of the effects of processing when they are by-products of the oil industry? Which of these products would be most acceptable to the child or the mother? In what order of nutritive value or priority should they be classified? FAO has produced a document[6] summarizing our knowledge of these new food sources, and pointing out the additional research that seems to us necessary. The question on which we in FAO would like to have your advice is the possibility of utilizing as human food products such as fishmeal, defatted soya meal, peanut meal, cotton-seed flour, sunflower seeds, sesame and, if possible, any other cheap protein-rich food.

KING: Are there any other cheap sources of protein that could be developed?

SCRIMSHAW: Coroso oil meal from the Coroso palm, which grows wild throughout a considerable part of tropical America, has been shown in chicken feeding to contain protein of higher biological value than either sesame oil or cotton-seed oil meal, and it seems to be very promising. Then, of the leaf meals, rami has a protein of good value and is a useful source of vitamins.

AUTRET: Coconut protein might also be added to the list. In the South Pacific region attempts are made to use it.

SCRIMSHAW: It may be that the residue from the African oil palm will also have value, at least for animal feeding. We are working on it.

AUTRET: If that is what we call in French "coprah", we have some information on it. It has been tried in France in animal feeding, but has been found definitely unsuitable for human feeding.

SCRIMSHAW: It has a very high protein content. I suggest that we need information on three things: availability, toxicity, and biological value. We might defer the question of availability, because what we are concerned with first is the suitability of these products as human food. As for toxicity, cotton-seed of course contains a toxic factor.

CATRON: Industry knows how to process cotton-seed now and remove the gossypol. We can feed it to pigs by adding chlorosulphate to counteract the effect of gossypol.

SCRIMSHAW: Sesame grown in Central America has a bitter aftertaste which makes it unsuitable, but this can be removed by solvent extraction. We don't know yet how that affects the biological value.

AYKROYD: Surely practically all these products have been fed in large quantities to animals?

CATRON: Yes.

DEAN: They have all been fed to man except coroso.

DARBY: That doesn't prove they're not toxic.

DEAN: No, but sesame, for instance, is an ordinary article of diet in some places in Africa.

DARBY: Yes, but the quantities needed to increase the protein intake significantly in young children may have quite a different effect from the amounts appearing in the diet under normal circumstances. However, that is an aspect we shall come on to later—the testing of these products in human subjects.

NATURE OF DEFICIENCIES

ROSE: I have hesitated to enter this discussion because I am merely a biochemist working in a laboratory, while most of you are on the firing line, trying to do the best you can under the circumstances with which you are confronted. But it does seem to me that we are off on the wrong track. We have already spent two or three days deciding what are the protein requirements, taking Professor Terroine's suggestion of expressing them in terms of a good-quality protein such as milk protein. We have agreed that for different age groups we must have a certain amount of this protein to meet reasonable minimal standards. Now it seems to me that we need to accept as a policy the expression of these requirements for different age groups in terms of the component amino-acids. After this, if we don't have the necessary information on the amino-acid intakes in those areas of the world where protein deficiency occurs, we must get it. The difference will show what must be done to bring current diets up to our proposed standards.

How can we discuss remedial measures until we know specifically what are the deficiencies in the different areas of the world? It might be that one supplementary protein would fit one area better than another; it might be that two or three would have to be combined, in different proportions, to accomplish the desired aim. But it seems to me that we have a clear programme to aim at. We cannot neglect amino-acids, no matter whether we are interested in them or

not. We must regard them as the active components, either free or in simple combinations. And we must know eventually what it is that people who are experiencing deficiencies fail to get, before we know what to do for them. In the meantime, while we are getting the necessary information, each person will have to do the best he can in the circumstances in which he lives.

FREMONT-SMITH: You have expressed, I think very beautifully, the long-range goal, and then you said, "in the meantime", and came back to the short-range goal. It seems to me appropriate for us to discuss what we should do in the meantime also, because this is a very practical consideration for some of the agencies concerned. But I hope we shall not lose sight of the long-range goal that you have in mind.

SCRIMSHAW: As Dr. Dean pointed out, the children with pre-kwashiorkor and kwashiorkor simply aren't getting enough protein of any kind. Almost any of these products alone or in combination, added to the diet of such a child, will prevent him from getting kwashiorkor, and presumably even help him to recover; so that for the immediate, practical, preventive aspect, we don't necessarily have to go into the details of amino-acid composition. But for improving the quality of diets that are already fairly high in protein of poor biological value, what you say becomes of vital importance.

ROSE: If any of these will prevent kwashiorkor, then use the one that is available, but I don't see how we can be very intelligent about it until we know what the deficiencies are.

RELATIVE BIOLOGICAL VALUES

KING: I think that what Dr. Rose has suggested is very basic and very clear as a guiding principle. It fits in completely with the pattern of our discussions in the first two sessions. I think we would all accept that as a guide for the future, but in the meantime we must make some practical appraisal of what to do in the next few years. This is a matter of urgency. Therefore I think it would be helpful if we make a rough relative rating of these products, always bearing in mind that in different areas different ones may be best.

MAYER: Can we really neglect what Dr. Rose said, even for the short term? For instance, the sulphur amino-acid content of sunflower seed is twice that of cotton-seed. If it becomes a matter of supplementing cereals, for example, I think that even for the short term, amino-acid composition should be the primary consideration.

KING: I was assuming that, Dr. Mayer. To try and get a practical rating for these products doesn't mean neglecting Dr. Rose's point, with which I am sure we are all in agreement.

AUTRET: The biological values of certain of these foods, as given in the FAO document,[6] are as follows:

Fishmeal	36–86
Soya-bean flour	65–92
Peanut presscake flour	56–59
Cotton-seed presscake flour	62–81
Sunflower-seed presscake flour	64

TERROINE: What exactly do you mean here by biological value? Is it the amount necessary for maintenance of the adult, or growth of the child, or is it a coefficient of retention?

ALTREI: I assume that the wide range of the figures in the table is because different authors have expressed the values in different ways.

KING: Dr. Catron, would you like to comment on the biological value of these products, not for stock-feeding but as human food?

CATRON: I have had experience with all of them in varying degrees, except coroso. For non-ruminants the number one oilmeal, as far as quality is concerned, is soya. Peanut probably would be second.

AYKROYD: What about the observations that you gave us yesterday on certain disadvantages of soya protein for young growing pigs in the very early stage of life? How do they fit in?

CATRON: Well, that might apply to all these foods, not only to soya. It appears that the baby pig has an incomplete enzyme system. I don't know whether or not that is true in the human infant, but I surmise that it might be. We know a great deal about most of those oilmeals for poultry, pigs and rats. I don't know whether or not Dr. Rose would agree with me, but if I had to rank them, from my own experience and from what I know in the literature, I would put soya first in quality. I am assuming adequate processing as regards time, temperature and moisture.

CRUICKSHANK: Dr. Allison has fed his animals, I think, on most of these things at one time or another.

ALLISON: We have used most of them, feeding them primarily as single sources of protein. I certainly agree with Dr. Catron that soya has the highest biological value, of about 75–80, while peanut flour has a value round about 60. I believe our approach should be to consider these proteins as supplements, and by examining their amino-acid patterns, make some predictions, as Dr. Rose has indicated, concerning their value as supplements to cereals. From our own analyses and from those in the FAO report,[6] all these presscakes would seem to be good supplements for cereals in varying degree. For instance, as Dr. Mayer pointed out, sunflower seed is unusually good because of its sulphur amino-acid content.

I think, therefore, that rating these protein sources for supplementation purposes I would put soya flour at the top, with peanut flour second. Sunflower would be valuable for its methionine and cystine, and if you could mix sunflower with peanut flour, I think you would have a good supplementation pattern.

ROSE: H. H. Mitchell found sunflower protein to be of a very high quality, even higher than soya flour.[15]

WATERLOW: It seems to me that these figures for biological value are of very little practical use, because they are for a food given alone, and not in combination with others.

SCRIMSHAW: Yes. All these products have a relatively high-protein content, and all of them will presumably be satisfactory in mixtures. The only real test is the experimental one.

WATERLOW: However, if we had data on their amino-acid composition, we should know more accurately which of these foods is likely to be a good supplement for any given staple.

TERROINE: I would like to lend my support to what has just been said. We really can't look at these products and classify them as absolute values in themselves. We have to consider them as supplements. I have given some particularly striking examples in my report. For instance, in one experiment rats were put on diets containing 1·7 per cent of nitrogen. When this nitrogen was all derived from peanut meal they gained 0·35 g. per day; but when 25 per cent of the

nitrogen was derived from sunflower seed and 75 per cent from peanut, they gained 1·35 g. per day—that is to say, nearly 4 times as much, although the total nitrogen intake was the same.

Dean: It might be useful if I summarized the results of some rat experiments in which the materials mentioned were used in supplementation.

Waterlow: Are these your own experiments?

Dean: No, they are a compilation from the literature.[16]

Soya is undoubtedly the plant protein about which we know most. A great deal of work can be summarized by saying that soya with cereals usually gives good results. The disadvantage is that it requires special cooking and cannot be given to people to cook in their own homes. It needs autoclaving, and sometimes debittering as well, but there are simple methods which can be used to give good results and have been used in my own laboratory.[17] The material so made has been fed with good effects to children suffering from kwashiorkor. It was, in fact, the first protein we used when we tried to substitute plant proteins for animal proteins.[18]

The chief disadvantage is the trypsin inhibitor, which I think must be removed, particularly if soya is used for diets of children. Work of the kind that Dr. Bengoa mentioned has, unfortunately, been spoilt because soya has been introduced into local dietaries without sufficient information on how it can and cannot be used. In America it is of enormous value in livestock feeding, and soya milks are on the market for the use of children who cannot tolerate cow's milk, because they are allergic to it. A manufacturing plant has been erected in Indonesia for the making of soya milk, using a proceess essentially the same as that used here, and the latest information about it is there are more technical problems than were first realized, and also that the product is going to be terribly expensive. The price of soya milk in the U.S.A. is also extremely high.

Peanut flour: the results of supplementation were as follows: peanut with maize, poor; peanut with rice, again, rather poor; peanut with oatmeal gave a biological value of 81 as against a calculated value of 77; peanut with wheat flour was as good as muscle-protein in promoting growth, but the amount of peanut-protein eaten to get the same effect was rather greater. Peanut with white flour was only about half as good as white flour with soya bean. In another experiment peanut with enriched wheat flour was not as good as skimmed milk.

Cotton-seed: with maize or white wheat flour the supplementary effect of cotton-seed was very poor; with flax-seed meal, poor; with lucerne, fairly good (biological value found: 68; calculated: 84); with soya bean, good (biological value found: 77; calculated: 60).

Cotton-seed, as has been mentioned, contains a toxic factor, gossypol. The animal experimenters have come to the conclusion that a certain amount of gossypol is not toxic and have defined the permissible limit. But cotton-seed also contains allergens which must be considered in human dietaries, although the effects do not seem to be very important. Cotton-seed has been used to supplement wheat flour.

King: Dr. Scrimshaw, didn't you get good results with cotton-seed and corn?

Scrimshaw: We got our best results with corn, sesame and coroso, and results that were satisfactory but not quite as good with cotton-seed, corn and coroso.

Dean: Cotton-seed oilmeal, a commercial product of this country given to me by the United States Department of Agriculture in New Orleans, showed in a few preliminary trials in my own laboratory that it was apparently

well-tolerated. It is bright yellow in colour, turning to brown when mixed with any other food, but it was perfectly well accepted.

Sesame: the only information I have is that sesame with soya bean had a protein efficiency ratio of 2·17; this was apparently a satisfactory combination. Autoclaved soya bean by itself had a protein efficiency ratio of 1·75. Sesame is an ordinary article in some dietaries in Africa and, I expect, elsewhere.

AUTRET: Yes, in Egypt and in China. However, intestinal trouble has been reported after the consumption of sesame presscake. This was attributed to an excess of calcium oxide.

DEAN: *Sunflower*: it has already been mentioned that sunflower seed is rich in sulphur amino-acids. It was considered to be a good supplement to maize, in a set of experiments on which I do not have full information. In the FAO report[6] a trial is mentioned in which sunflower seed was fed to 130 children, with apparently good results. There is a danger that sunflower-seed meal contains a lot of silica, which might be detrimental. More work is required before it can be considered suitable for human nutrition. I myself have given it to a few children with no apparent ill-effects, but cannot consider my work in any way definitive.

Coconut: poor, in combination with maize, and quite good in another experiment with yellow maize.

KING: Dr. Salcedo in the Philippines reports that they are having good results in children with a coconut milk emulsion made from the presscake.

DEAN: *Flax-seed* with rye gave a good result, better than with 8 per cent of milk protein. With maize it was very poor.

Coroso: nothing known. That is all the information I have.

CRUICKSHANK: These biological results are very much in keeping with the results predicted theoretically from the amino-acid composition, as outlined by Dr. Rose. It might therefore be very valuable to prepare our mixtures on that theoretical basis.

DEAN: I don't think any of us would deny that that is the best theoretical basis, but we might well be surprised at the results of biological experiments, which do not always follow expectation.

AUTRET: Quite right.

ROSE: Certainly allowance would have to be made for the availability of amino-acids in these products.

GYORGY: I would prefer experiments in which the diet used commonly in the country in question was given to animals with and without supplement. I think this is the only reasonable basis for drawing the conclusion we are aiming at from animal experiments. In many instances the basal diet may be slightly enriched with some protein in order to cover the protein requirement of a fast-growing animal such as the rat. This enrichment alone should not be sufficient to produce growth without further supplementation.

KING: You are willing to undertake these?

GYORGY: I have been doing this for over two years.

GOPALAN: From the available evidence, the diets of children in under-developed communities seem to be deficient in lysine, methionine and leucine. I do not know the essential amino-acid composition of all the foods discussed here. If it is true that sunflower has the highest concentration of sulphur-containing amino-acids, that would be the thing to which the highest priority would have to be given.

AYKROYD: Has nobody ever tried supplementing the poor rice diet with sunflower?

GOPALAN: Not to my knowledge.

SCRIMSHAW: We are just exploring rice plus sunflower now.

PLATT: The real problem, I should have thought, would be to improve diets based on roots and tubers like cassava.

AUTRET: I don't know if you can improve or supplement the value of cassava. The amino-acid content of cassava is very low, and the distribution of these amino-acids rather unfortunate. Therefore you cannot speak of "supplementation".

AYKROYD: There are some experiments on record showing the results of supplementing cassava with groundnuts and other foods.[19] The rat dies on the cassava diet alone. If you add any other source of protein, it will survive, but it will not grow well.

AUTRET: I think we have plenty of information on the supplementary value of all these products, when added to the basic diet of many regions. This aspect has been covered in a recent survey.[6]

PRACTICAL EXPERIENCE WITH MIXTURES

AUTRET (continued): After considering the biological value of these products as such and their amino-acid composition, we should try to build a suitable combination of amino-acids to meet the requirements. A great many of the presscakes are deficient in lysine, and in some others the limiting factor is methionine. It may be possible to achieve a good nutritive value by a combination of basic foods with a mixture of presscake flour. It may also be necessary to add a certain amount of fishmeal or even skimmed milk to the mixture. In a paper we have prepared[20] we tried in a tentative way to build up a suitable mixture from fish flour, peanut flour, and pulses. At the International Nutrition Congress at Amsterdam in 1954 we put forward a similar scheme for supplementing rice, maize and wheat with pulses, fishmeal, and dried skimmed milk.[21] These are just examples of how to start.

A good many preliminary trials of these supplementary foods have been made in different parts of the world. The results, as far as we know them, are summarized in the FAO paper.[6] Perhaps others here have practical experience of feeding these products to human beings.

SÉNÉCAL: We have used a flour made of peanut mixed with millet, which is the basic food in French West Africa. We analysed the amino-acid content of both peanut and millet flour, and found that for most amino-acids the composition of the mixture was satisfactory, a deficiency in one cereal being made good by the other; this, however, was not the case with methionine, which is low in both millet and peanut. We fed this flour to children in hospital under strict medical supervision, first in small amounts and then increasing slowly over as long a period as possible, because if you only do it for a few days, the results do not show much; in fact, very often other symptoms may develop only after a certain length of time.

We were able to follow some children for several weeks. The peanut flour was mixed with millet flour in the proportion of 10, 20, and even 30 per cent. We gave an average of 25 g. a day for one month to children whose average age was 6 months. They gained about 40 g. a day. The tolerance in all cases was good, and there were no ill-effects such as vomiting or diarrhoea; in some children the results achieved were better than those we obtained with fish flour. We haven't yet compared the peanut-millet mixture with milk alone. This is now being done.

We also gave some older children 50 g. of this flour every day for 55 days. Their average weight gain over that period was 2·17 kg.—that is, a gain of 40 g. a day.

In another native centre we tried this mixture as a supplement in 116 small children, of whom some were still being breast-fed and others had been weaned. Here again the growth-rate was normal. Therefore I can only give my impression that this mixture did nothing to prevent a normal rate of growth.

In the stage of pre-kwashiorkor weight is stationary, or only increases very slowly. We have been able to re-establish satisfactory growth by adding these mixtures to the diet. It is my impression that we got better results when some fish flour was added to the millet–peanut mixture. At the beginning the mixtures were made up into a pap with milk; it was only later, when the mothers brought the children regularly, that we were able to make them up with water only.

These experiments are continuing, and we hope to elaborate stricter criteria for the future. I also hope to make use of the method advocated by Professor Terroine; that is to say, to calculate the coefficient of nitrogen retention, which we have not been able to do so far.

GOPALAN: In India extensive trials have been made with soya milk, groundnut cake, and various other vegetable protein foods. The Indian Council of Medical Research is shortly publishing a special report entitled "Milk Substitutes", embodying all this work. I do not speak about it in detail because I was not personally concerned in these trials. Moreover, from a practical point of view neither soya nor groundnut are suitable for use on a large scale, soya because it cannot be produced as a milk, and groundnut cake because it is considered more valuable to feed it to animals. This brings up the question of availability. Theoretically many of these presscakes may be suitable for man, but in practice it may be better to convert them to milk or meat through the cow or pig.

DEAN: At the Jamaica conference I reported the results of feeding a soya preparation to infants with kwashiorkor. However, these trials were carried out under rather special conditions.

AUTRET: All the tests of these various products that I know of are of rather short duration. It is important to consider what harmful effects they might have on animals or human beings in the long run.

POSSIBLE RISKS

GYORGY: I entirely agree with that, and I would like to give an example. More and more in the United States the diagnosis of allergy against cow's milk is made very early, and in my opinion erroneously made, by my colleagues in practice. The infants are put on soya-bean milk for a very long time, often for a year or two. The usual supplements are given after 4 or 5 months, especially cereals. Still, I have now seen a great number of very bad cases of dystrophy and anaemia in infants fed only soya milk. Similar observations were reported recently in a paper in one of the paediatric journals.[22]

Recently I have seen a 3-year-old child with a weight of 13 lb. (!) kept on soya milk as the sole source of food. All these observations may be exceptions. However, it is a fact that we have not enough reliable data on the long-term use of soya milk as the sole source of protein in infants. In consequence I would like to disagree with one statement in the excellent report "On Protein Supplements for Children" as prepared by the Nutrition Division of FAO.[6] I have the sentence on page 22 in mind: ". . . The nutritive value of soya bean milk and cow's milk, when used as the sole source of protein, was closely comparable".

KING: That is exactly the kind of problem on which I have been trying to sound a precautionary note. It is far more fundamental, I think, than has been fully faced in this conference.

HOLT: I agree with Dr. Gyorgy that the great majority of children who are thought to be cow's milk-sensitive are not, and the diagnosis is made erroneously. But there are some who are cow's milk-sensitive, and when those children are put on soya milk, they sometimes become soya bean-sensitive too, and that may explain some of these untoward results which occasionally one sees with soya bean.

CRUICKSHANK: What is the incidence of this unfortunate reaction? Is it known approximately how many children in the United States are getting large quantities of this soya milk, and is the incidence of bad results very low or moderately high? Any product that is to be introduced on a large scale may carry a certain risk, and we must know what that risk is and be prepared to take it, provided it is very small.

GYORGY: That is a public health problem, and what I said, as you know, was a clinical impression. When we get patients now with very bad malnutrition, I make a diagnosis *a priori* that this child has been on soya-bean milk, and I hit the diagnosis in an unusually large proportion. But how many children are kept on soya bean and for how long, I don't know.

In addition to that, as Aubertin and his associates have shown,[23] in animal experiments soya bean is harmful when given in large amounts as the sole source of protein. In dogs it produces severe hepatic injury with necrosis.

When we embark on the introduction of a food like soya milk, we should have sufficient experimental and biological data on its *long-term* use, which as yet we don't have. We have not been able to collect clinical data. I shall speak more about this later, when we consider in detail the testing of new products used for infant feeding.

DARBY: These difficulties have occurred with products which are being used as the sole infant food. But at the moment we are focusing our discussion primarily on substances which will be used as supplements. Therefore the infant may not be so completely dependent on the food. This does not to my mind decrease the necessity of our being vigilant, both in preliminary testing and in continued observation, but it does put us in a slightly different position. Moreover, we must not necessarily assume that the effect of soya milk is connected with its protein. It may be something else which is lacking or has disappeared from the soya milk.

We must look at this matter in perspective. In the Indonesian plant, for instance, an attempt is being made to produce an infant food which will be better than the diet currently used that results in kwashiorkor. It may not be the ideal as yet, but it may still be an improvement. We must continue to strive toward the ideal, and watch carefully what happens. But the fact that it does not answer all the problems may not mean that it is entirely bad.

CRUICKSHANK: I think we must keep the very practical aspect of this problem before us, that there are probably dozens of people dying every day from lack of protein. If, with all necessary reservations, we can introduce something on a large scale which will improve the lot of these people, we are taking a practical step forward, and we have to take the risk of running into difficulties; as we run into them, we must smooth them out. For that reason I think it would be a mistake to concentrate for too long on the possible theoretical dangers that might result from the use of these substances. Let's get on with a well

safeguarded, practical production of a protein substitute or supplement, and decide now on the criteria which we think it ought to pass.

KING: With regard to soya, several large milling companies with highly qualified research personnel have tried to use for human food the finest quality soya presscake that they could manufacture. The record to date indicates that there has been something in soya bean that mitigates against continued public acceptance. I think that, apart from possible ill-effects, the problem of *acceptance* is very serious and should be looked at realistically in *any* study of this kind.

PLATT: Experience in China with soya bean is entirely different from that which the Chairman has described. In the United States, when the soya bean is used for human food, it is used chiefly in the form of soya flour. The soya bean has in fact been handled in the two countries in entirely different ways. I believe the Chinese techniques provide a basis for research: we should find out what the Chinese have done with the soya bean so that it has retained its place in their food economy since the days of Shen Nung who is said to have introduced it along with four other main crops nearly 6,000 years ago.

One thing which is not generally known is that soya milk has been introduced recently—in the early thirties—for the feeding of human infants and by Western-trained physicians. In the record I got together in 1938[24] of foods that were used for feeding infants, there was only one instance of soya bean being fed to a human baby as soya milk. Soya curd, on the other hand, is given to their young children by Chinese mothers, who have experience beyond ours in these matters.

AUTRET: This soya milk was used by the Chinese only in order to prepare curd immediately afterwards.

PLATT: Except by these Western-trained physicians, such as Ernest Tso, who introduced it into China in 1913.

HOLT: John Ruhrah used it in this country.

PLATT: I am talking about the introduction of soya milk into China. We are apt to think that this is a product with which the Chinese have had experience over the ages, but it is not.

DEAN: Your own experiences with it were bad, weren't they?

PLATT: Yes, both babies to whom I fed it died.

DEAN: Dr. Darby made a remark which I think was a debating point, about the use of soya either as a partial or a total food. Surely most of the children described by Dr. Gyorgy who are given soya milk instead of cow's milk, get other food as well? If they do, then soya is not the only source of protein, and in fact it is being used in circumstances rather similar to those in which we are advocating its use in under-developed countries. My point is, is it not premature to set up a plant of this kind in a place like Indonesia? Doesn't that seem to be an extremely solemn responsibility?

FREMONT-SMITH: It is very important that we try to get all the facts we can—both actual facts and opinions or theories from competent people—and then try to see them in perspective. We should have no hesitation in saying what we really feel or know to be the case. I can see very well, in many public health measures, that one may have to go forward with a calculated risk. The important thing is that the risk be as specifically stated and as carefully guarded against as can be, particularly when the United Nations agencies are taking the responsibility. I think we should make every effort to make clear the potential risks and the unknowns, so that if you have to say afterwards: "This turns out to be not so good as we thought", you have laid the groundwork for retaining the confidence of the people whom you are trying to approach with these products.

SCRIMSHAW: I think we should emphasize a distinction between the use of soya as an infant milk, which has been criticized, and its use in mixed feeding in the diets of pre-school and older children, of which there is much successful experience. In the programmes in Central America it has been given over a period of 3 years in a mixed supplement, with very satisfactory results. That has been reported from many parts of the world, so that soya is certainly a very useful protein.

RECOMMENDATIONS

AYKROYD: For my part, I should like to relate this discussion to the practical steps that should be taken at the present time by the international organizations —WHO, FAO and UNICEF.

We are all agreed that there is an urgent need to supplement the sources of protein in the diet in many of the under-developed countries, particularly when the staple food is manioc or banana rather than cereals. The value of any supplement must be considered in the light of its protein and amino-acid content, and that of the local diet to which it will be added. In addition, more animal and human experiments are needed, because the chemical rating does not always tell the whole story.

The concentrates that we have been discussing are possible sources of supplementary protein. We are perhaps justified in encouraging, with due caution, the use of these foods. Since these may need to be processed, very thorough testing will be necessary before they can be distributed on a large scale. This is a problem to which Dr. Gyorgy has given much thought, and which we must now discuss in detail. I urge, however, that the necessary testing should be reduced to a minimum, so that too much time is not wasted before practical action can be taken. UNICEF is ready to supply funds for the construction of plants to produce some of these protein-rich foods, but is less willing to support testing or development work needed at an earlier stage.

BORCIC: The Executive Board of UNICEF has always expressed a certain reluctance to allocate funds for research, field experiments, and pilot plants in connection with these special foods. The reason for this is the fact that UNICEF is not a technical UN agency, but an agency to help countries in the practical development of some of these schemes, with the aim of improving the nutritional state of particular groups, such as mothers and children. For technical advice we look on the one hand to WHO and on the other hand to FAO. This group, as I see it, is more or less in a position to advise our technical advisers.

If we take as an example the Indonesian plant for making soya-bean milk, this plant has an economic background; the country was importing large quantities of milk, mostly for institutional use, and as a supplement in feeding schemes in schools, in health centres, maternity and child centres, and hospitals. Soya is locally available, although the product after the necessary processing will be expensive, just as expensive as the imported milk or even a little more so. But it still is an economic gain to the country, because all these countries are chronically short of foreign currency.

What we want now is guidance on practical methods of extending assistance to these countries, to help them to start producing supplementary protein foods, particularly for the children and the mothers. We have already been approached by some of the countries to help them in producing fishmeal powder for their particularly vulnerable groups, including schoolchildren. We have also been approached by certain countries to see if we can help them to use cotton-seed

for the same purpose. Moreover, UNICEF has so far spent over ten million dollars in various countries, with the aim of solving the problem of protein deficiency that results from lack of milk.

KING: Is there any provision for an alert watch being kept, and for maintaining close touch with the people using the soya-bean milk plant, so that we can be sure there is no cumulative bad effect from feeding large amounts of soya milk to babies?

BORCIC: The plant is still the property of UNICEF, but as far as I understand, it is the intention of the Institute of Nutrition in Djakarta to check the results and to do control experiments on infants. When it comes to children of school age it is much less important, because this is a relatively small supplement to their diet.

PLATT: If we raised the age level at which these supplements were given, we would immediately have a safeguard, and that is what I have recommended. Any cow's milk preparations of any kind should be reserved for the first year of life. There are very few places where you can't get some milk, and if it were reserved primarily for that first year, then we needn't worry quite so much about what happens after that.

FREMONT-SMITH: Would that be in place of making carefully controlled studies during the first year? Isn't it important also to have careful studies under hospital conditions?

PLATT: I don't think we need necessarily stop doing that work, but I am suggesting stepping up the age at which products of this kind are first given as a safeguard in recommending what should be done immediately.

FREMONT-SMITH: But not in place of the experimental studies?

PLATT: As an interim measure until the results of these studies are clear.

SCRIMSHAW: I think Dr. Gyorgy would agree that there are three levels: first the infant, with whom you have to take rather elaborate precautions; secondly the pre-school child, with whom you must still be very careful, because he may be getting a considerable amount of the supplementary mixture; and lastly the school child, for whom the supplement may be an incidental thing, in addition to his usual diet, and therefore in most cases can be given almost immediately without elaborate precautions.

The Testing of Processed Protein-rich Foods

GYORGY: I accept Dr. Scrimshaw's three levels. However, I think, as Dr. Burgess suggested, we should consider this whole question of testing in much more detail. At the beginning of this session I made the dogmatic statement that any food used in young infants has to be looked upon as a drug, if it is introduced into the country as a new supplement and has not been used before. One might argue that milk does not require any special investigation. We all know that milk is our most valuable food product, and it would certainly be audacious on my part to say that we should look upon it as a drug. However, if you take skimmed milk and introduce it into a tropical country, and a drum of skimmed milk stands at a temperature of 100° F. for weeks and months, I defy anybody to assert that six months or a year later it is just as good as the milk that we drink here in New Jersey. Therefore even with milk the question of storage immediately raises important problems. But I don't wish to discuss skimmed milk, because we are here concerned with other food products containing protein which are available or can be introduced cheaply into underdeveloped countries.

In what I have to say I shall lean heavily on the note on protein supplements for children prepared by the Nutrition Division of FAO,[6] and I shall point out a few minor discrepancies in our views. The question is, if we have a supplement X, for instance fish flour—and I mention fish flour only as *one* example—what criteria must it fulfil? Here again I am very fortunate, because the Joint WHO/FAO Expert Committee on Nutrition at their fourth meeting in Geneva in 1954 has already spelled out in principle the necessary criteria that I also had in mind. I would like to quote from their report:[1]

"Certain steps and investigations are needed in the development of any food preparation for these purposes (supplementary feeding). Knowledge of its nutritive values through chemical analysis and biological analysis is essential, and the product must be acceptable and low in cost. The possibilities for its large-scale production and for its storage, preservation and distribution must be evaluated. It is of the utmost importance that a new preparation should be proved to be harmless by suitable tests on animals, and that control studies of its value when given to children be made before it is recommended for wide-spread use. After the product has been introduced into general use, continuing observations should be made as to its suitability and its value for nutrition of the child."

I have, in principle, nothing to add to this paragraph, and I congratulate Professor Platt, who, as Chairman of the Expert Committee, must take the responsibility for this statement.

TESTING PROCEDURE

Now to go into detail: fish protein is an excellent protein; there is no question about that. I would not hesitate to give fresh fish to young infants in this country, although it is rarely done. But when we get to fish flour, we are dealing with processed food—that is, with an industrial product. The great majority of food producers try to supply the best product possible, but when we are dealing with the health of infants we must be very strict. We have to write our own specifications, and these specifications are that we must know what kind of fish and what part of the fish went into the flour, what possible contaminants were added, knowingly or unknowingly, and where there are contaminants like sand or the contents of the intestinal tract of the fish, what was the process of collection. Was the fish very fresh, or had it been standing and become putrid?

Then, when we know about the fish that goes into the plant, we next have to know the process. We must know exactly what extracting substances or other extraneous chemicals have been used. Recently, for instance, ethylene oxide is being used for the preservation of food, and we know that this is toxic. Extracting agents such as trichlorethylene might be used, which may be toxic if not completely eliminated. There are possibilities of interaction between extracting fluids and constituents of the fish flour or other protein supplement, which may produce harmful chemical compounds.

Secondly, we have to know whether and to what extent the product was exposed to heat and oxygen. Many data are available in the survey compiled by the FAO staff[6]; I might, for instance, refer to page 6, where it is stated that drum-dried fish, heated for 180 minutes, loses 85 per cent or more of its available lysine. Obviously we hope that the fish we are talking about will not be exposed to such heat, which in only a short space of time might perhaps be important in starting a chain reaction between carbohydrate and protein. Evaporated milk stored at a high temperature, e.g. 100° F., all the year round or even for part of

the year, may after 12–24 months show a very large reduction in biological value, and a complete loss of growth-promoting activity for rats. At the same time analysis shows a sharp drop in the content of various essential amino-acids.[25, 26]

Such changes occur even under sterile conditions. Therefore after the collection, preparation and processing of the food comes the first chemical analysis, with special reference to amino-acids. The product must also be tested bacteriologically and the vitamin composition should be determined. All these points are brought out in the FAO report. Then there is the very important problem, also emphasized by the joint Expert Committee, of storage. There is no way of assessing the harmful effect of storage except by retesting the product after a period of time. I don't want to go too specifically into technical detail as to how to do this; I am just writing the headlines, so to speak. The same tests should be repeated after 3 months, and again after one year of storage. Perhaps accelerated storage conditions, that is to say, artificially produced tropical conditions, might be used, as in the U.S. Army laboratories.

The question is, how often should these tests be done? Should they be done on each batch, and in each country where the product is being used? No. With any new process they should be done before the product is introduced. If the same process is being used in another country, then, perhaps, it would not be necessary to do a very intensive investigation. Perhaps sometimes a few batches should be taken off the shelf and tested, as the Food and Drug Administration does in this country even with vitamins.

After the first chemical and bacteriological analysis, biological assessment should be done by suitable tests on animals—I quote the words "suitable tests on animals" from the Expert Committee report. At the first introduction of a product prepared by a new process I would like to see at least two species of animals used, with perhaps a few of a third species thrown in. This recommendation is based on the experience of the Food and Drug Administration in Washington, who have found that some toxic materials can be detected in one species but not in another. If the process is changed, then that is a new product. Or if the process is changed in minor details, then some expert should advise whether it is necessary to do complete retesting.

DEAN: I remember how, when I investigated the present production of one of your soya flours in this country, I found that the last biological tests on children had been done several years previously, and since then there had been a large number of modifications in the process.

WATERLOW: When you say that the tests should be repeated, do you mean the tests of toxicity or the tests of nutritional value?

GYORGY: Both. The nutritive value of the food product might have been reduced, and harmful substances might somehow have crept in during processing. We know that there are toxic substances in cotton-seed and in soya bean. With fishmeal it is improbable, but we cannot deal with improbabilities, we have to deal with facts. Any processing may produce substances that are harmful. Therefore when we go to animal experiments with a food product, we are accomplishing a twofold purpose—to test its nutritive value, and to test its range of harmlessness.

It is succinctly and correctly stated in the FAO report[6] that "In general, such foods would be used as supplements; namely, they would be given in relatively small daily amounts, their proteins supplementing those already provided by the diet". Therefore I agree that the harm resulting from a small reduction in

biological value is not as important as it would be if the processed product constituted the only food given to the infant.

After the first biological tests, which should be of acute and sub-acute duration – sub-acute meaning, perhaps, 5 months in rats – if the results are satisfactory, the product is ready for testing on infants and children. I fully agree with Dr. Autret that, even before anything is done or any expense incurred, the product in question should be given to mothers to test acceptance, if they do accept it, we should give it next to older children as supplements, and if everything is satisfactory, we may then give it to young children down to about one year, again as a supplementary food. The protein value can be assessed in accordance with the deliberations of the previous two sessions, on the basis of essential amino-acids, and the amount necessary can then be calculated. For the tests on young children the product has to be given in hospital, under the direct surveillance of paediatricians. I insist on this, which I think is most important.

Apart from the question of acceptability, I personally do not feel that it is necessary to do any special tests on humans, except to follow the general clinical condition together with the usual measurements, such as serum protein levels and some enzymes in the blood. Liver function tests may be done, but no metabolic studies, in my opinion, are essential. These studies must be continued, perhaps, for 2 or 3 months, and when the results appear to be satisfactory, the food should be distributed on an out-patient basis.

Finally, the new product must be given to at least two hospitals, one in an under-developed country, and the other in a more highly developed country, where nursing supervision is simpler. Clinicians are always slightly biased, but two groups will probably not be similarly biased. Of course, as I said before the product has to be retested after 3 months' storage, and again after a year.

Because of its practical importance I discussed this whole programme of testing with a small subcommittee of the conference, consisting of Drs. Darby, Cruickshank, Holt, Autret and myself, and we were in complete accord. The schedule I have outlined, and on which we are all agreed, can best be summarized in a table (Table 39).

KING: In considering the testing procedure, I think we should recognize two distinct needs—foods for intensive use at the stage when an infant would normally be on a whole milk diet, and foods for use during the transitional period, when children are adjusting themselves to the family type of mixed diet.

GYORGY: Yes, we should make this distinction based on common sense.

AYKROYD: Surely it is not assumed that the entire existing literature or, I might almost say, libraries on this particular subject and on these groups of foods, will be ignored? Take peanut meal, for example: you will find in the literature, going back over a considerable number of years, an enormous number of experiments indicating that peanut meal in general has a certain biological value which makes it appropriate for use under certain circumstances. I take it that our problem is to decide whether any particular preparation of peanut that may be advocated has received treatment which might alter its biological value.

GYORGY: Quite right.

FREMONT-SMITH: But isn't it implicit in what has been said that if a new process has been used, you can't just estimate whether or not there might have been some alteration, but you actually have to go through the testing procedure?

SEBRELL: I would like to emphasize that. No one should embark on a clinical trial of a new product in which a new process or a new extraction technique of

TABLE 39

Procedure for testing a new processed food

1. Raw material, on which we must know:
   A. Its nature.
   B. Its source.

2. Processing, on which we must know:
   A. Temperature and duration of heating.
   B. Exposure to moisture.
   C. Nature of solvents used for extraction.

3. Bacteriology:
   A. The product should be free from pathogens, including B. coli and Clostridia.
   B. It should have the usual low count of micro-organisms.

4. Chemical analysis:
   A. Nitrogen.
   B. Amino-acids.
   C. Fat.
   D. Carbohydrate.
   E. Ash.
   F. Selected vitamins.

5. Storage:
   The product should be retested after 3 and 12 months, after storage under the conditions in which the food would be used in the field.

6. Animal tests:
   A. Acute tests on rats (4 to 6 weeks).
   B. Sub-acute tests (5 months).
   C. Tests on one species of larger animals—baby pigs or puppies.

7. Tolerance and acceptability trials on adults for 1 month.

8. Trials on healthy children, or hospitalized healthy children.

9. Preliminary clinical tests on control groups of malnourished individuals in regional hospitals.

10. Field studies.

The chemical analyses and the animal tests can be conducted concurrently. The acceptability trials on adults (No. 7) may be begun before these tests are concluded. The trials on infants and children (Nos. 8 and 9) cannot be begun until all the previous tests have proved satisfactory.

some kind has been tried, without the most thorough pharmacological, toxicological, chemical and animal experimentation, to examine whether this new process or scheme has in any way made the product toxic or deleterious or injured its protein value. It seems to me very important that we include this word of caution, that with any new process that is introduced, large-scale field trials should not be attempted until careful laboratory experiments have been done.

DEAN: Dr. Gyorgy, has dried skimmed milk been sufficiently tested so that it might be excluded from the category of new foods?

GYORGY: Yes. If the dried milk is of good quality, and not long stored. If it has been stored for 2 years in the tropics, then I don't consider it as absolutely safe.

DEAN: Even with dried skimmed milk, you cannot answer a question like this in one word, can you?

GYORGY: Yes, you can, because *fresh* dried skimmed milk, processed, does not need any testing.

AYKROYD: We could ask the representative of UNICEF how many million tons have been distributed around the world with apparently no ill-effects. Could you give us figures, Dr. Borcic?

BORCIC: An average of about 100 million pounds a year.

DEAN: With no ill-effects?

BORCIC: None.

FREMONT-SMITH: I think we ought to be cautious about that. We don't mean to imply that in fact there have been careful tests for ill-effects on all those million pounds.

AYKROYD: Ill-effects may have cropped up here and there among the millions of benefiting children.

BURGESS: Some milk that was distributed to the Arab refugees in Jerusalem —although not by UNICEF—was stated to have caused a good deal of diarrhoea. No definite cause was found: it probably was old stock which had deteriorated. It is quite true that many of these things are done, and there is no field check on what actually happens.

SCRIMSHAW: Similar reactions have occasionally cropped up in Central America, but they have not been serious.

FREMONT-SMITH: With all the wonderful work that has been done, it is unfortunate that field tests have not been able to provide a clear statement about the harmlessness of all the dried milk that has been distributed.

EFFECT OF PROCESSING

DARBY: I think Dr. Dean's point is that, even when we talk about a widely-used and accepted product X, both its source and the nature of the processing to which it has been subjected must be defined and identified in exactly the way we have been discussing, since important differences can easily mask themselves behind a common name. As an example, I might refer to a recent paper[27] describing an investigation into the nutritive value for growing rats of the proteins of beef, egg, milk and wheat germ, after these foods had been extracted with various solvents. Briefly, the findings were as follows: the nutritive value of beef for growing rats was found to be markedly reduced by simultaneous dehydration and defatting with ethylene chloride or propylene, under certain conditions, and the reason for this was that methionine was rendered unavailable or the methionine content was decreased. Growth could be improved by adding methionine. This reaction was peculiar to beef. The same procedure exerted no deleterious effect on several other proteins. From a nutritional standpoint, extraction of beef with pentane or trichlorethylene was found to be perfectly satisfactory.

I quote from the authors this next statement: "A given solvent may exert a deleterious effect upon a specific product and only under certain conditions. Therefore generalizations concerning the influence of any processing method upon the nutritive value of foods cannot be made safely without experimentation."

Now, it happens that one of the solvents of which these authors approved—trichlorethylene—when applied to soya and perhaps other products, has led to a widespread epidemic of very serious disease among cattle and other livestock. My point therefore is that, regardless of how common a product is as an article of commerce, one sample may not be identical with another from a different source, although in name they may be the same.

CATRON: At Iowa State College, where trichlorethylene extraction of oilmeals originated, we have had some very sad experiences, as Dr. Darby indicated. From personal experience I wish to emphasize that the effect of every new solvent used in the processing of a food should be tested with many species—not two or three, but several. Trichlorethylene meal was satisfactory for chicks, and large quantities can be given to baby pigs, so that it seems to be harmless in an animal stomach. But it is fatal to cattle, and unfortunately it was allowed to slip by without adequate testing in ruminants. Nearly thirty plants had been established, and they are all closed down today. Therefore I can't emphasize too much the necessity for careful testing of every process, not only extraction, although that is especially important, but all processes that are used on different types of protein. The tests should be done on several different species.

DARBY: A great deal of testing and a great deal of study are certainly essential initially, and some kind of control is necessary on any continuing process, but I don't think this involves repeated animal and human tests every few months. I want to get this clear, as otherwise with any processed material it would be absolutely impossible to keep the plant going.

DEAN: You are absolutely right, Dr. Darby, provided you know everything about a product, but I believe that you cannot disregard the necessity for continuous testing. We are dealing with materials about which much is unknown.

I know of a marine food that is being produced as a commercial venture. Its biological value has been found to vary extremely widely, for reasons that the manufacturer cannot ascertain at the moment. His own idea is that the variation has nothing to do with the processing and is not even due to the kind of fish used as raw material. He thinks it may be due to the fact that at certain times of the year the fish feed on material which alters their constitution, probably by enzymatic action, in such a way as to cause deterioration. The alteration is not detectable by ordinary means, I am sure; otherwise the manufacturer would not have allowed the deteriorated batches to go for biological testing. He has come to the conclusion that the difficulties of standardization are so great that he is probably going to abandon the whole thing. He cannot compete with dried skimmed milk, which costs very little more than his fish product.

AUTRET: There are enzymes in fish that destroy the amino-acids, but slowly. They are active at higher temperatures, and you have to go below 0° C. to destroy this enzymatic activity completely. But the fishing industry now knows how to reduce this activity.

DARBY: Even the animal-husbandry people, dealing with relatively well standardized processes, find discrepancies creeping in which they must restudy.

CATRON: That is correct. But in industry, quality control sees that the product is maintained at the original standard at which it was offered. After major changes in the method of processing, then, naturally, a complete survey of all the methods and techniques of testing has to be carried out. The result is that we get a standardized product. Dr. Dean apparently does not have a standardized product. That, as I know very well, is one of the big problems in fishmeal production.

HOLT: I have been very much interested in Dr. Autret's observations, and in what others have done with the various commercial fishmeals, which are more or less discard preparations for feeding animals. What we should do is to make use of the technical know-how of the American *human* food manufacturer, instead of picking up this discard material from the animal food business. The manufacturers do know how to make acceptable and safe food products, and I hope that Dr. Autret and others will take advantage of their knowledge.

DEAN: They may know in relation to a particular batch of raw material, but our problem is to make use of the material available in the countries where we are working. We nearly always find that the only fish available are fish which will not be eaten by the local population, and we are always concerned with the price of the finished product.

HOLT: The price depends on distribution. American ventures have failed because the products weren't widely enough distributed. But the technical problems have been solved for at least four kinds of fish—cod, redfish, mackerel and tuna.

DEAN: All of which are quite high in price or fetch good prices in sophisticated markets.

HOLT: But I would be much surprised if the same techniques could not be applied to fish that are available off the coast of Africa.

LIMITATIONS OF ANIMAL TESTS

GOPALAN: Theoretically, I don't think exception could be taken to these recommendations, but quite often even with all this battery of tests we do run up against difficulties. I am thinking more of tests of nutritional value rather than tests of toxicity. For example, I recall two instances recently from Indian experience. Some five years ago there was a fairly severe shortage of rice, and a suggestion was made by a responsible worker that 25 per cent of the rice in the poor rice diet could be safely replaced by cassava. He carried out some experiments on animals, and showed that in a poor rice diet, by replacing 25 per cent of the rice with cassava, although the protein level of the diet was still further reduced, the animals actually grew better. That was a very puzzling finding, so the experiment was repeated in three or four laboratories, and the same result was obtained. What had happened was that in the poor rice diet the limiting factor seemed to be calcium, and cassava evidently supplied additional calcium.

Then, since we thought that growth was a fallacious criterion, a study was made of liver histology in three different laboratories. At the end of this "sub-acute experiment" the livers were sent to three pathologists, who did not know from which batches the samples came. The curious thing was that out of three people, two said there was no difference, and one actually said that the livers on the rice diet were worse. We didn't know what to do about these observations, because they were quite contrary to all the conclusions one draws from the literature. Then we decided that these experiments were not enough, and that we should continue them to three generations.

That was one type of fallacy into which animal experiments could lead us. There was another case in which hydrogenated vegetable oils were tested on rats in one laboratory, and the conclusion was drawn that after continued feeding of these oils the third generation of rats developed cataract or blindness or other ocular lesions. That started a controversy which raged for 5 years, and for a while the vegetable oil industry was very much upset. There was no

unanimity. We really didn't know what significance could be attached to these animal experiments, which were carried out under well controlled conditions in one of the institutes of animal nutrition. The experiment had to be repeated in three different laboratories, and the results disproved the original conclusion.

FEEDING TRIALS WITH CHILDREN

GOPALAN (continued): What I am trying to say is that, in the choice and trial of these foods, even with all this battery of tests we can't be certain of being *completely* out of danger. I think we have to approach the problem from several directions: after animal tests and analyses we still have to rely on feeding trials with sick children, and on field studies in which the products are given to borderline cases and normal subjects. It would be wrong to develop a sense of security on the basis of animal experiments alone, since in them we do meet difficulties of the type I have just described.

VERHOESTRAETE: Is the general idea that the products we have been discussing will be used essentially as supplementary foods, or are they also intended for clinical use in the treatment of kwashiorkor? I see no reason why milk should not continue to be the basis of treatment in hospital, whereas these other products are to be used essentially in a broad programme for the prevention of kwashiorkor.

GOPALAN: I think the distinction between clinical cure and prevention is not very clear-cut when you work in tropical regions. When you speak of prevention, I suppose you are thinking of distributing a product to the population at a given time. At any given time you will have children who are just 6 months old, children who are 2 years, children who are 5 years. It may well be that with children who are 6 months old you can talk of prevention, in the sense that they are having breast milk and have not so far developed any degree of protein malnutrition. Therefore the product will be used not only for prevention, but also for mitigation and cure.

The use of these preparations in the treatment of malnourished subjects has been advocated not so much as a means of treatment, but as a means of getting information about their value for prevention. I think this is a valid and useful approach for people working in under-developed regions. Perhaps it is a more economical and direct approach even than animal experiments, in spite of the practical and theoretical objections.

WATERLOW: Although in theory I entirely agree with you, the trouble is that such trials are technically extremely difficult. I had experience of this when I was working 10 years ago on the effect of lipotropic factors in kwashiorkor. It is not the ideal method of testing a food to give it to these sick children, and it hardly gives the product a fair chance. The clinical trials of milk and casein mixtures described by Dr. Hansen are an excellent example; I thought they were very well carried out, and the number of patients was large, but even so his conclusions were questioned, and he probably would not claim that the results were absolutely clear-cut.

GOPALAN: I agree that to test a product in a case of kwashiorkor may be weighting the scales against it, but I think that is really erring on the safe side.

I should also like to point out that it is dangerous enough to apply the results of experiments with normal rats to normal human beings; it is even more dangerous to apply them to sick human beings. Therefore I think it is an economic and satisfactory way of testing a product, to try out its effectiveness in the treatment of kwashiorkor. Malnourished children are preferable in this

respect to normal children, because the scope for improvement is greater. In the discussion on Dr. Hansen's results I perhaps gave the impression that I was critical of that approach; far from it, only I think we must be more sure of the therapeutic criteria that we use. In our own work, where we have studied the effect of treatment with skimmed milk in 50 cases of kwashiorkor, and with Bengal gram and rice in 30 cases, we have tried to use a battery of clinical and biochemical criteria, such as the disappearance of oedema, the time taken for a certain increase in weight and in serum albumin level, the time taken for disappearance of diarrhoea, etc. By using these criteria, we think we can say which line of treatment is really effective, and we can apply that information to the problem of prevention. I think this approach is extremely useful in under-developed countries, and perhaps more valid even than animal experiments.

WATERLOW: I am inclined to think that it would be better to carry out these tests on healthy or nearly healthy children under strictly controlled conditions, as you yourself suggested a little while ago.

SÉNÉCAL: I agree that it is preferable to carry out the experiments on healthy children, especially if they can be assembled in hospital under very rigorous clinical observation. But there is in itself a paradox between the words, "healthy" and "hospital", and this applies particularly to under-developed countries where there is great pressure on hospitals. There is also the risk of infection, and a further difficulty is that such a long period of time is required for any feeding trials. Lastly, living conditions in a hospital are not the child's normal conditions, and this detracts from the rigour of the test.

Consequently, I think we have to carry out experiments on sick children, and I agree especially with what Dr. Gopalan said, that the results obtained by means of such experiments are valid and applicable. If a certain product can cure cases of kwashiorkor, in my opinion it can certainly prevent the occurrence of the disease.

AUTRET: Would you start the trials on children in hospital as soon as you have the results of the first experiments on animals?

GYORGY: Yes.

BORCIC: It has been many times stated at this meeting, more or less axiomatically, that there is chronic protein deficiency on a global scale. The whole discussion is dominated by the pathology of the small child. However, UNICEF is also interested in the other groups, such as older children of pre-school age, the school child, and pregnant and nursing mothers, all of whom have a chronic dietary deficiency of protein. I should like to ask for guidance in the criteria to be laid down for these groups as well.

DEAN: It is in small children that the problems of protein deficiency tend to be most acute, and the results that we get with them can almost certainly be applied without further test to older children.

There is the further point it is almost impossible, in practice, to limit the use of a food which is specifically labelled "not for the youngest children" but which, in fact, is being used for the youngest children, with the result that in some centres in South Africa it has caused disease. I think that is a further part of our responsibility; we cannot limit the use of a food with any certainty to one group, and therefore it must be harmless to all.

SCRIMSHAW: Dr. Borcic wasn't here when growth curves were presented which showed without exception that in various under-developed areas the retardation in growth and the effects of malnutrition came between the period of weaning and school age; in some cases at least the school children did not

show retardation in growth, but grew at a rate parallel to that of well-nourished children: so I think all of us have a sense of urgency about the pre-school child.

CATRON: It seems to me that if a product is tested in the infant or young child, it should certainly be satisfactory for the adult. I would like to emphasize that if animal tests are used, it is wise to use young animals.

PLATT: But your own observations showed that soya protein in the first weeks of life was not good for pig growth, yet I am sure you would not exclude it from the pig later on in life, so the application of the test to the very young infant has its limitations.

CATRON: But it is much better to know that if it is not going to work in the very young pig, it might not work in the very young infant—if that is true. I don't know whether or not it is true.

EMERGENCIES AND LONG-TERM PROJECTS

BORCIC: One practical problem we are faced with is this: we have now been approached by a government to help them in using a by-product of an industry, namely fishmeal, which is available in the country in large quantities, to supplement the diet of the pre-school and school child. The plan is to add the fishmeal in bread or gruel or whatever other food is prepared for the children. The industry could increase its present output of processed fish, and the government is ready to take over the whole output of fishmeal for this purpose.

Must we now start this whole battery of tests from A to Z, before anything can be set on foot, or can we find a short-cut in certain ways? I am not speaking of bacteriological and toxicity tests, which, of course, have to be done, but of the clinical testing of this product. Can there be a short-cut in that particular respect?

GYORGY: Dr. Borcic, may I call your attention to Table 39, paragraph 7, p. 165, where it is stated that trials may be begun on adults for tolerance and acceptability even before sub-acute animal experiments are finished. Therefore, you can begin 6 weeks after the product has been made available. Everybody agreed that a new product, with a new process, has to be fully tested, and 6 weeks will not cause any real delay.

DEAN: The important point is to prevent the erection of an industrial plant on a large scale unless all possible precautions have been taken beforehand.

MAYER: I wonder if Dr. Gyorgy would volunteer any comments on another problem which crops up fairly frequently: when the introduction of a new food product is necessary because of an emergency situation, would there be any choice among the various tests, when time is very much of the essence, as to which you think the most essential?

GYORGY: Well, with any product, you can but use common sense and balance the risk against the emergency.

SÉNÉCAL: Dr. Mayer is right in saying that when a new product is being tried there are two approaches. One is the approach of the laboratory man who is trying to minimize any possible risk by carrying out all the analyses and trials that are necessary; and there is also the approach of the doctor in the field, who sees malnutrition, disease and death around him, and who sometimes has to act very quickly in order to prevent worse things from beginning. That problem is an extremely acute one, especially in under-developed countries. I think that the doctors in the field should on such occasions be allowed to use the product with the minimum of tests, and I would suggest the two following: crude chemical analysis, without going into all the details of amino-acids, and also a crude analysis of toxicity and bacteriological content.

We don't know, of course, when we are giving a new product, what symptoms, if any, may develop. An example is the production of coeliac disease by wheat gluten. Some effects may develop years later. But, after all, it is a risk which we have to weigh and take, balancing it with what would happen if we did not give the product. Whatever the value of the various tests made with animals, I feel that clinical trials, in the long run, will give the most valid and the most conclusive results.

I suggest that we might alter the recommendations put forward by Dr. Gyorgy in one respect, by saying that we could start giving the product to adults and even to children before the experiments with animals are completely finished.

Terroine: I think any divergence that exists can easily be solved if we realize that the problem can be approached from two different angles. You can approach it from the point of view of urgency, and from the point of view of long-term projects, especially those that require new industrial installations.

I think we have enough information from work in the past to enable us, in the circumstances described by Dr. Sénécal, to take action as doctors without having to carry out further studies. But when we have to set up new industries, then, of course, further studies are necessary, especially since all industrial undertakings are now dependent very much on each other. For instance, in the case of groundnut oil, the old method of pressure is being replaced more and more by new chemical methods that ensure the maximum extraction of oil. These chemical methods, of course, may bring about very great changes in the composition of the final product, and that is where the tests and the analyses indicated by Dr. Gyorgy become absolutely essential.

Bengoa: There is a very interesting example that occurred in Spain in 1939, at the time of the Civil War. Because of the lack of foods rich in protein, they decided to use a legume which, presumably, would supply protein of good quality. This legume was Lathyrus, and the resulting lathyrism which occurred had catastrophic effects in Madrid. The important point I want to make is that the usual tests carried out by Professor Diaz failed to reveal toxicity in animals. He believes that there is a fat-soluble factor which is toxic to man when consumed in large quantities, but not to animals.

Fremont-Smith: It has been clearly brought out that no testing will give absolute safety. No matter how careful we are, there is the possibility that we will run into a catastrophic situation every once in a while. I think the points we are trying to make are (1), that every reasonable effort should be made to avoid this possibility, and (2), that if such a disaster should take place, it can be shown that those who are responsible for introducing the processed product have taken every possible step to protect the public, and incidentally to protect themselves.

Cruickshank: I think we all realize that risks may be involved, particularly in the long-term use of these substances. Above all, we must not be lulled into a false sense of security by trials on animals and men that have been carried out over relatively short periods. It is obviously important to make careful observations in the field of the results of these feeding programmes over a period of years.

Fremont-Smith: Apart from the physical damage that might be caused by a bad product, there is the psychological damage. It is one thing if a child gets hurt by food from the local market—food which is prepared locally and is part of the cultural tradition. It is quite another story if this food carries the stamp of a country with a high technological development, if it is introduced

from another land with the promise that it is going to be good, and then it turns out that it wasn't adequately tested. This could produce serious trouble and misunderstanding among peoples.

ACCEPTABILITY

FREMONT-SMITH (continued): That brings me back to the point made by Dr. Burgess at the beginning, that however desirable and well-tested a food may be, it is no good if it is not accepted. If it once fails, it can put the programme back for years. Therefore the people who make the preliminary studies of acceptance should be very well trained in health education and in the local culture and tradition.

HOLT: I should like to give an example that shows the need for caution in field trials, and illustrates the problem of acceptance. This is a story that Dr. Ylppö of Finland told me about the introduction of dried milk immediately after the war. Dried milk was a complete novelty in Finland. At the close of the war a large shipment was sent in by UNRRA, the distribution of which was Dr. Ylppö's responsibility. The shipment came to Helsinki in 5 lb. cans, and it was distributed in these containers throughout the country. The reports that came back were very unfavourable: "it makes the children sick"; "they get diarrhoea"; "they don't like it"; "it is spoiled". Ylppö immediately recalled all the milk to Helsinki. It was taken out of the 5 lb. tins and repacked under his direction into small packages. On these packages there was nothing to indicate that it was milk, but it was labelled, "Special Baby Food from the United Nations", with the picture of a smiling baby on the outside. The product was sent back to the various centres. This time the reports were all enthusiastic: "this is wonderful"; "we have never had such a good packaged food for Finland's babies". Editorials even appeared in the local papers, "why can't all of Finland's babies have this wonderful new baby food from the UN?" (Laughter.)

I know of another example, relating in this case to fish flour, but without the same happy ending. A certain firm, after considerable research, produced a codfish product for infants. We tested it thoroughly in our clinic for acceptability and made observations on weight gain, nitrogen retention and plasma proteins. We tested it continuously and intermittently on the most sensitive infants—prematures, and it gave excellent results. The technical problems were solved; the problem of shelf stability was solved; the problem of acceptability by the baby was solved; but the product did not "take" in the American market. This may have been due to the marketing procedure. Whatever the cause, the product was abandoned after a trial of about a year.

BURGESS: Another example is given in Margaret Mead's book on cultural attitudes.[28] She records the consumption of canned milk in Burma, where milk itself is not acceptable; because the can had two birds on the outside and had apparently nothing to do with milk, it was accepted.

FREMONT-SMITH: That is the way in which cultural anthropological knowledge can help in the problem of changing food habits.

PLATT: An important point that has come out of the comments made round the table is the emphasis on developments in agriculture and the improvements of economic, social and cultural conditions, as contrasted with the attention devoted to by-products of industry. This is a matter about which some of us feel strongly. That it has been so expressed is, I think, most reassuring and we should not lose sight of our convictions in considering in retrospect what we have been discussing during the conference.

KING: We have been searching for agreement—and in a large measure I think we have found it—on the orderly development of a procedure for the introduction, sponsorship and use of new foods or new processed products. Apart from the problem of safeguarding health, this procedure requires consideration of economics and of social and cultural pattern, as Professor Platt has just reminded us. These things are very complex, but if the results are successful they will be of great benefit to humanity.

A special responsibility falls on the United Nation's organizations, WHO, FAO, and UNICEF, and I hope that they may have been able to derive some help and guidance from these discussions.

Summary

The task of filling the gap between protein supplies and protein needs is one that must be tackled in many different ways. Much work has been done and is being done by the Food and Agriculture Organization and by individual governments; the conference therefore confined itself to certain aspects of this vast problem. It was taken for granted that every effort is being made to increase the production of milk and livestock, but since in many parts of the world this can only make a small contribution to the total in the foreseeable future, other sources of protein have to be considered. The obvious protein foods capable of immediate development are vegetable products of various kinds, and fish. Time did not allow the discussion of sources of protein that are still in a highly experimental stage, such as algae or moulds, nor was any attention given to the possibility of using synthetically manufactured supplements.

From a practical point of view there are two complementary lines of attack: the first—the regional approach—is to encourage the production and consumption in each part of the world of the foods most valuable as a supplement to the local staple. The second is by the production on an industrial scale of what in agricultural circles are known as protein concentrates. These can be manufactured wherever convenient, and distributed on a world-wide basis.

Before going into details the conference devoted some discussion to general principles. Measures of both types, to be effective, depend on an accurate knowledge of the amino-acid composition of foods. In *Rose*'s words, "the only logical way to select the best product for supplementary feeding is to accumulate information on the amino-acid distribution in the diets actually consumed . . . we must know eventually what it is that people who are experiencing deficiencies fail to get, before we know what to do for them". It is, of course, implicit in this statement that we have some criteria, however approximate, of what constitutes deficiency.

Since the analytical methods are difficult, and tend to give variable results in different hands, *Terroine* proposed that the creation of a central laboratory for amino-acid analysis might be a useful contribution. This suggestion was not approved by all, since it would bring with it the risk of discouraging local effort.

The next general problem is that of evaluating the effectiveness of any supplement that might be chosen. Terroine emphasized that chemical analysis alone is not enough; we are dealing not with pure proteins but with foods, and therefore the product must be tested biologically in the way it will be used—as a supplement to other foodstuffs. Lastly, as *Scrimshaw* pointed out, any protein-rich food that is developed either locally or industrially must fulfil other criteria besides being biologically effective: it must be safe, cheap, easy to store, and above all, acceptable.

The conference then went on to consider examples of what is being done along these lines in different parts of the world. In India and in Guatemala a great deal of information has been collected about the amino-acid intakes of various age groups, and the amino-acid composition of the foods they eat. This information was used in devising supplementary mixtures of locally grown foodstuffs rich in protein, that are now being tested in animals and in man.

Although the problem must in the main be tackled on a regional basis, it is to be expected that the solutions found in different parts of the world will have something in common and will depend on the same broad categories of foodstuffs. The best vegetable sources of protein are legumes, seeds and green leaves.

Dean reviewed briefly the available information about some of these—soya bean, groundnut, sunflower seed, cotton-seed, and coconut. The figures quoted for biological value show a very wide scatter, and little meaning can be attached to them, since in many cases the foodstuffs have been tested as the sole source of protein, or else in combination with staples such as wheat with which they would not be used in practice. The soya bean heads the list, and has been far the most studied because of its extensive and successful use in animal feeding. As a food for man soya presents certain problems: it is difficult to prepare in an acceptable form, and for this reason attempts to introduce it as a supplementary source of protein have in some cases been a failure—an example of the need for caution emphasized by Scrimshaw. *Platt* pointed out that the methods of preparation developed by the Chinese have centuries of tradition behind them, and cannot be introduced overnight into a new environment.

Although the development of local food resources must be the first line of attack in bridging the gap between requirements and supplies, a useful contribution can be made, particularly to the needs of the more vulnerable groups, by converting to human use materials rich in protein that at present are either wasted or used for animal feeding. One such possible source is fishmeal; another is the residue from oil seeds that is left behind after the oil has been extracted. This is a problem that has engaged the attention of FAO for some time. Several kinds of fish flour have been produced, and tests have been begun under their auspices. In India feeding trials are being carried out with meal or flour made from groundnut presscake. Such projects, if successful, are clearly capable of wide extension, without in fact causing serious competition between animals and man.

Products of this kind have to be processed on an industrial scale. If they are to be fed to human beings, and particularly to young children, it must be shown without a shadow of doubt that they are not only effective but safe. This involves careful and repeated control of the industrial process—of factors such as the possible toxic effects of solvents, or the destruction of amino-acids by heat. It also involves stringent testing of the final product. *Gyorgy* outlined a schedule of tests that any product should pass before it can be considered safe for distribution. This includes chemical and bacteriological analysis, testing of shelf-life under various climatic conditions, biological tests on several species of animals in both short- and long-term experiments, and finally trials on human beings, including undernourished children. From many points of view it would clearly be a disaster if a product of this kind, distributed perhaps by one of the international agencies, were found to be unsatisfactory or harmful. A small subcommittee, headed by Gyorgy, was therefore appointed to formulate the testing procedure in detail and to report to the conference as a whole.

Even when a product has been found to be both effective and safe, this is not the end of the matter. If it is to be introduced into a community from outside, it is essential to take account of cultural and psychological factors, in which food-habits are deeply rooted. Otherwise, however good the intentions, the whole enterprise may founder.

References

[1] JOINT FAO/WHO EXPERT COMMITTEE ON NUTRITION. Fourth Report (1955). *Tech. Rep. Wld Hlth Org.*, No. 97. Geneva.

[2] BROCK, J. F. and AUTRET, M. (1952). Kwashiorkor in Africa. *F.A.O. nutr. Stud.*, No. 8, Rome. *W.H.O. Monograph Series*, No. 8, Geneva.

[3] SECOND INTER-AFRICAN CONFERENCE ON NUTRITION (1954). Malnutrition in African mothers, infants and young children. Report of Conference held in Gambia, 1952. Ed. B. S. Platt. H.M. Stationery Office, London.

[4] TROWELL, H. C., DAVIES, J. N. P. and DEAN, R. F. A. (1954). *Kwashiorkor*. Edward Arnold, London.

[5] CONFERENCE ON PROTEIN MALNUTRITION (1955). Proceedings of a Conference held in Jamaica, 1953. Ed. J. C. Waterlow. University Press, Cambridge.

[6] FOOD AND AGRICULTURE ORGANIZATION (1955). Protein supplements for children. Mimeographed note by the Nutrition Division. Unpublished.

[7] TERROINE, E. F. (1956). Les besoins protéiques de l'homme et les conditions de leur satisfaction. *Bull. méd. A.O.F.* In the press.

[8] BALASUBRAMANIAN, S. C., RAMACHANDRAN, M., VISWANATHA, T. and DE, S. S. (1952). Amino-acid composition of Indian foodstuffs. *Indian J. med. Res.*, **40**, 73, 219.

[9] VIJAYARAGHAVAN, P. K. and SRINIVASAN, P. R. (1953). Essential amino acid composition of some common Indian pulses. *J. Nutr.*, **51**, 261.

[10] PHANSALKAR and RAMACHANDRAN (1956). *Indian J. med. Res.*, to be published.

[11] AGUIRRE, F., ROBLES, C. E. and SCRIMSHAW, N. S. (1953). The nutritive value of Central American corns. II. Lysine and methionine content of 23 varieties in Guatemala. *Food Res.*, **18**, 268.

[12] AGUIRRE, F., BRESSANI, R. and SCRIMSHAW, N. S. (1953). The nutritive value of Central American corns. III. Tryptophan, niacin, thiamine and riboflavin content of 23 varieties in Guatemala. *Food Res.*, **18**, 273.

[13] SCRIMSHAW, N. S. and SQUIBB, R. L. (1952). Agricultural implications of the protein nutrition problem. *Turrialba*, **2**, 44.

[14] SQUIBB, R. L., DIAZ, F., FUENTES, A., GUZMÁN, M. and SCRIMSHAW, N. S. (1952). The relation of forages to nutrition problems in the American tropics. *Proc. 6th Int. Grassld Congr.*, **2**, 1544.

[15] MITCHELL, H. H., HAMILTON, T. S. and BEADLES, J. R. (1945). The importance of commercial processing for protein value of food products; soybean, coconut and sunflower seed. *J. Nutr.*, **29**, 13.

[16] DEAN, R. F. A. (1953). Plant proteins in child feeding. *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 279. H.M. Stationery Office, London.

[17] DEAN, R. F. A. (1954). Preparation of soya bean and banana diet. In *Report of Second Inter-African (C.C.T.A.) Conference on Nutrition, Gambia*, 1952. p. 279. H.M. Stationery Office, London.

[18] DEAN, R. F. A. (1952). Treatment of kwashiorkor with milk and vegetable proteins. *Brit. med. J.*, **2**, 791.

[19] LADELL, W. S. S. and PHILLIPS, P. G. (1955). Groundnut flour as a source of protein in the Nigerian diet. A report to the West African Council for Medical Research from the Hot Climate Physiological Research Unit, Oshodi, Nigeria. Unpublished.

[20] FOOD AND AGRICULTURE ORGANIZATION (1956). A survey of protein-rich foods. Mimeographed report by the Nutrition Division. Unpublished.

[21] AUTRET, M. and VAN VEEN, A. G. (1955). Possible sources of proteins for child feeding in under-developed countries. *Voeding*, **16**, 286.

[22] NISENSON, A. (1955). Hypoproteinemia and edema in eczema. *J. Pediat.*, **46**, 544.

[23] AUBERTIN, E., RIVIÈRE, J., LOISEAU, P., DEBOT, P. and MALINEAU, R. (1954). Effect of high plant protein regimens on the liver of dogs. *Amer. J. clin. Nutr.*, **2**, 413.

[24] PLATT, B. S. and GIN, S. Y. (1938). Chinese methods of infant feeding and nursing. *Arch. Dis. Childh.*, **13**, 343.

[25] HODSON, A. Z. (1952). Nutritive value of milk proteins; stability during sterilization and storage of evaporated milk as determined by the rat repletion method. *Food Res.*, **17**, 168.

[26] HODSON, A. Z. (1954). Nutritive value of milk proteins; stability during sterilization of evaporated milk as determined by the rat-growth method. *Food Res.*, **19**, 224.

[27] CLARK, H. E., HOOPER, A. S. and MCCORD, M. L. (1955). The nutritive value of the proteins of beef extracted with different solvents, and of egg, milk and wheat germ for the growing rat. *J. Nutr.*, **55**, 63.

[28] MEAD, M. (1953). *Cultural Patterns and Technical Change*. UNESCO, Paris.

APPENDIX I: DAILY PROTEIN INTAKES AND PERCENTAGE OF CALORIES REPRESENTED BY PROTEIN, SUMMARIZED FROM PUBLISHED STUDIES

(From a Working Paper on Protein Requirements prepared by T. R. Davis and J. Mayer, Nutrition Department, Harvard School of Public Health, on behalf of FAO.)

Age group	Author	Year	Optimal protein intake		Per cent calories
			g./day	g./Kg./day	
0–1 years	Wang	1928	32	4·0	16
Mean weight: 8 Kg.	Stearns	1939	27	3·4	13
Calorie allowance: 800	Holt	1940	32	4·0	16
	Levine	1945	32	4·0	16
1–3 years	Molchanova	1936	48	4·0	16
Mean weight: 12 Kg.	Roubstein	1936	42	3·5	14
Calorie allowance: 1,200	Leitch and Duckworth	1937	4·8	4·0	16
	Clements	1946	50	4·2	17
	Clements (1946) also gave a minimal requirement of 2·3 g. per Kg. per day (9 per cent of calories).				
3–4 years	Daniels	1935	51	3·2	14
Mean weight: 16 Kg.	Leitch and Duckworth	1937	60	3·8	17
Calorie allowance: 1,400	Hawks	1938	64	4·0	18
4–5 years	Daniels	1935	54	3·2	14
Mean weight: 17 Kg.	Leitch and Duckworth	1937	60	3·5	15
Calorie allowance: 1,580	Maroney	1937	60	3·5	15
	Hawks	1938	68	4·0	17
	Farr	1938	53	3·1	14
5–6 years	Leitch and Duckworth	1937	64	3·2	16
Mean weight: 20 Kg.	Maroney	1937	62	3·1	15
Calorie allowance: 1,640	Hawks	1938	80	4·0	20
6–7 years	Holt	1921	60	2·6	13
Mean weight: 23 Kg.	Leitch and Duckworth	1937	69	3·0	15
Calorie allowance: 1,800	Maroney	1937	67	2·9	15
	Eppright	1954	53	2·3	12
7–8 years	Leitch and Duckworth	1937	68	2·7	14
Mean weight: 25 Kg.	Maroney	1937	70	2·8	15
Calorie allowance: 1,900	Eppright	1954	58	2·3	12
8–9 years	Wait	1933	56	2·0	11
Mean weight: 28 Kg.	Molchanova	1936	78	2·8	15
Calorie allowance: 2,100	Leitch and Duckworth	1937	70	2·5	13
	Maroney	1937	78	2·8	15
	Eppright	1954	64	2·3	12
9–10 years	Wait	1933	65	2·1	12
Mean weight: 31 Kg.	Bjeloussoff	1935	93	3·0	17
Calorie allowance: 2,200	Leitch and Duckworth	1937	78	2·5	14
	Maroney	1937	84	2·7	15
	Eppright	1954	65	2·1	12

Age group	Author	Year	Optimal protein intake		Per cent calories
			g./day	g./Kg./day	
10–11 years Mean weight: 33 Kg. Calorie allowance: 2,350	Hasse	1882	73	2·2	12
	Herbst	1898	46	1·4	8
	Sundström	1911	63	1·9	11
	Müller	1914	76	2·3	13
	Tigerstedt	1916	79	2·4	14
	Holt	1921	76	2·3	13
	Wait	1933	66	2·0	11
	Leitch and Duckworth	1937	83	2·5	14
	Maroney	1937	92	2·8	15
	Eppright	1954	69	2·1	12
11–12 years Mean weight: 36 Kg. Calorie allowance: 2,600	Hasse	1882	79	2·2	12
	Müller	1914	79	2·2	12
	Tigerstedt	1916	50	1·4	8
	Holt	1921	83	2·3	13
	Sherman	1920	97	2·7	15
	Richet	1928	54	1·5	8
	Wait	1933	72	2·0	11
	Leitch and Duckworth	1937	90	2·5	14
	Maroney	1937	101	2·8	15
	Eppright	1954	79	2·2	12
12–13 years Mean weight: 40 Kg. Calorie allowance: 2,840	Herbst	1898	60	1·5	8
	Müller	1914	80	2·0	11
	Tigerstedt	1916	108	2·7	15
	Holt	1921	76	1·9	11
	Sherman	1920	84	2·1	12
	Wait	1933	64	1·6	9
	Leitch and Duckworth	1937	100	2·5	14
	Maroney	1937	104	2·6	15
	Eppright	1954	84	2·1	12
13–14 years Mean weight: 45 Kg. Calorie allowance: 3,100	Sundström	1911	99	2·2	13
	Müller	1914	81	1·8	10
	Tigerstedt	1916	99	2·2	13
	Holt	1921	77	1·7	10
	Sherman	1920	90	2·0	12
	Leitch and Duckworth	1937	117	2·6	15
	Maroney	1937	117	2·6	15
	Eppright	1954	104	2·3	13
14–15 years Mean weight: 50 Kg. Calorie allowance: 3,300	Herbst	1898	70	1·4	8
	Tigerstedt	1916	100	2·0	12
	Holt	1921	70	1·4	8
	Wait	1933	65	1·3	8
	Leitch and Duckworth	1937	130	2·6	16
	Maroney	1937	125	2·5	15
	Eppright	1954	100	2·0	12

Age group	Author	Year	Optimal protein intake g./day	Optimal protein intake g./Kg./day	Per cent calories
15–16 years	Sundström	1911	103	1·8	12
Mean weight: 57 Kg.	Tigerstedt	1916	114	2·0	13
Calorie allowance: 3,500	Holt	1921	120	2·1	14
	Wait	1933	86	1·5	10
	Leitch and Duckworth	1937	148	2·6	17
	Eppright	1954	103	1·8	12
16–17 years	Sundström	1911	108	1·8	12
Mean weight: 60 Kg.	Tigerstedt	1916	102	1·7	12
Calorie allowance: 3,550	Wait	1933	66	1·1	8
	Leitch and Duckworth	1937	150	2·5	17
	Eppright	1954	102	1·7	12
17–18 years	Tigerstedt	1916	93	1·5	10
Mean weight: 62 Kg.	Holt	1921	124	2·0	14
Calorie allowance: 3,600	Wait	1933	93	1·5	10
	Leitch and Duckworth	1937	124	2·0	14
	Eppright	1954	105	1·7	12
18–19 years	Leitch and Duckworth	1937	96	1·5	11
Mean weight: 64 Kg.	Eppright	1954	102	1·6	12
Calorie allowance: 3,600					
19–20 years	Eppright	1954	98	1·5	11
Mean weight: 65 Kg.					
Calorie allowance: 3,600					

Age group	Author	Year	Protein intake g./day	Protein intake g./Kg./day Optimal	Protein intake g./Kg./day Minimal	Per cent calories
20–30 years	Sherman	1920	44	0·7		5·6
Mean weight: 65 Kg.	Leitch and Duckworth	1937	50	0·8		6·4
Calorie allowance: 3,200	Hegsted, Stare *et al.*	1946	50	0·8		6·4
	Stare	1945	25		0·4	3·2
	Harte	1947	25		0·4	3·2
	Rose	1949	25		0·4	3·2
	Bricker	1949	40	0·6		5·4
	NRC	1953	65	1·0		8·8
	Leverton	1954	20		0·3	2·4
	Hegsted	1955	25		0·4	3·2

References to Appendix I

BJELOUSSOFF, W. A. (1953). *Problems of Nutrition*, Moscow, **4**, 78.
BRICKER, M. L., SHIVELY, R. F., SMITH, J. M., MITCHELL, H. H. and HAMILTON, T. S. (1949). *J. Nutr.*, **37**, 163.
CLEMENTS, F. W. (1946). *Med. J. Aust.*, **2**, 404.
DANIELS, A. L., HUTTON, M. L., KNOTT, E. M., WRIGHT, O. E., EVERSON, G. J. and SCOULAR, F. (1935). *J. Nutr.*, **9**, 91.
EPPRIGHT, E. S., SIDWELL, V. D. and SWANSON, P. P. (1954). *J. Nutr.*, **54**, 371.
FARR, L. E. (1938). *Amer. Med. J. Sci.*, **195**, 70.
HARTE, R. A. and TRAVERS, J. J. (1947). *Science*, **105**, 15.
HASSE, S. (1882). *Zeitschr. f. Biol.*, **18**, 553.
HAWKS, J. E., BRAY, M. M. and DYE, M. (1938). *J. Nutr.*, **15**, 125.
HEGSTED, D. M., TSONGAS, A. G., ABBOTT, D. B. and STARE, F. J. (1946). *J. Lab. clin. Med.*, **31**, 261.
HEGSTED, D. M. *et al.*, in press.
HERBST, O. (1898). *Jb. Kinderheilk.*, **46**, 245.
HOLT, L. E. and FALES, H. L. (1921). *Amer. J. Dis. Child.*, **22**, 371.
HOLT, L. E., Jr. and MCINTOSH, R. (1940). *Holt's Diseases of Infancy and Childhood*. pp. 14–177. New York.
LEITCH, I. and DUCKWORTH, J. (1937). *Nutr. Abstr. and Rev.*, **7**, 257.
LEVERTON, R. M. (1954). *The Amino-acid Requirements of Man*, p. 55. The National Vitamin Foundation, Inc., New York.
LEVINE, S. Z. (1945). *J. Amer. med. Ass.*, **128**, 283.
MARONEY, J. W. and JOHNSTON, J. W. (1937). *Amer. J. Dis. Child.*, **54**, 29.
MOLCHANOVA, O. P., IVESDKAYA, I. M., RYSKINA, E. B. and POLTEVA, YU, K. (1936). *Vopiosy Pitariya* (*U.S.S.R.*), **5**, 103.
MÜLLER, F. (1914). *Zentrabl. f. Physiol.*, **28**, 749.
NATIONAL RESEARCH COUNCIL, FOOD AND NUTRITION BOARD, U.S.A. (1953). Publication No. 302.
ROSE, W. C. (1949). *Fed. Proc.*, **8**, 546.
ROUBSTEIN, L., BIRGER, L. and FREID, R. (1936). *Bull. Biol. Med. Exp.* (*U.S.S.R.*), **1**, 235.
SHERMAN, H. C., GILLETT, L. H. and OSTERBERG, E. (1920). *J. biol. Chem.*, **41**, 97.
STARE, F. J., HEGSTED, D. M. and MCKIBBIN, J. M. (1945). *Ann. Rev. Biochem.*, **14**, 431.
STEARNS, C. (1939). *Physiol. Revs.*, **19**, 415.
SUNDSTRÖM. (1911). *Skand. Arch. Physiol.*, **24**, 97.
TIGERSTEDT, C. (1916). *Skand. Arch. Physiol.*, **34**, 151.
WAIT, B. and ROBERTS, L. J. (1933). *J. Amer. Diet. Ass.*, **8**, 403.
WANG, C. C., HAWKS, J. E. and HAYS, S. B. (1928). *Amer. J. Dis. Child.*, **35**, 968.

APPENDIX II: EXISTING RECOMMENDATIONS FOR DIETARY ALLOWANCES

TABLE 1

Food and Nutrition Board, National Research Council
Recommended Daily Dietary Allowances, Revised* 1953

Designed for the maintenance of good nutrition of healthy persons in the U.S.A.
(Allowances are considered to apply to persons normally vigorous and living in temperate climate)

	Age years	Weight kg. (lb.)	Height cm. (in.)	Calories	Protein gm.
Men	25	65 (143)	170 (67)	3,200†	65
	45	65 (143)	170 (67)	2,900	65
	65	65 (143)	170 (67)	2,600	65
Women	25	55 (121)	157 (62)	2,300†	55
	45	55 (121)	157 (62)	2,100	55
	65	55 (121)	157 (62)	1,800	55
	Pregnant (3rd trimester)			Add 400	80
	Lactating (850 ml. daily)			Add 1,000	100
Infants‡	months				
	0–1§				
	1–3	6 (13)	60 (24)	kg. × 120	kg. × 3·5‡
	4–9	9 (20)	70 (28)	kg. × 110	kg. × 3·5‡
	10–12	10 (22)	75 (30)	kg. × 100	kg. × 3·5‡
Children	years				
	1–3	12 (27)	87 (34)	1,200	40
	4–6	18 (40)	109 (43)	1,600	50
	7–9	27 (59)	129 (51)	2,000	60
Boys	10–12	35 (78)	144 (57)	2,500	70
	13–15	49 (108)	163 (64)	3,200	85
	16–20	63 (139)	175 (69)	3,800	100
Girls	10–12	36 (79)	144 (57)	2,300	70
	13–15	49 (108)	160 (63)	2,500	80
	16–20	54 (120)	162 (64)	2,400	75

* In planning practical dietaries, the recommended allowances can be attained with a variety of common foods which will also provide other nutrient requirements less well known; the allowance levels are considered to cover individual variations among normal persons as they live in the United States subjected to ordinary environmental stresses.

† These caloric recommendations apply to the degree of activity for the reference man and woman.

‡ The recommendations for infants pertain to nutrients derived primarily from cow's milk. If the milk from which the protein is derived is human milk or has been treated to render it more digestible, the allowance may be in the range of 2–3 gm. per kg. There should be no question that human milk is a desirable source of nutrients for infants even though it may not provide the levels recommended for certain nutrients.

§ During the first month of life, desirable allowances for many nutrients are dependent upon maturation of excretory and endocrine functions. Therefore no specific recommendations are given.

Table 1 is reproduced by kind permission of the National Academy of Sciences, National Research Council, from their publication No. 302: *Recommended Dietary Allowances*, Revised 1953.

TABLE 2

Protein Requirements For Growth

(Within age groups the larger allowance applies to the younger child.)

Age group	Protein (gm. per day)	
	per kg.	per lb.
Infants—to the end of the 2nd year	4·0–3·5	1·8–1·6
Children—2–3–4 years	3·0–2·5	1·3–1·1
5–15 years	2·0–1·5	0·9–0·7

TABLE 3

Daily requirements of juveniles

Category	Body weight (lb.)	Approximate equivalent age (years)	Requirements per day	
			Calories	Protein (gm.)
Boys	20	1	1,050	35
	30	3	1,300	37
	40	5	1,600	40
	50	7	1,850	45
	60	9	2,100	50
	70	10	2,300	55
	80	12	2,500	60
	90	13	2,650	65
	100	14	2,850	70
	110	15	3,000	80
Girls	20	1	1,050	35
	30	3	1,300	37
	40	5	1,600	40
	50	7	1,850	45
	60	9	2,100	50
	70	10	2,300	55
	80	11	2,450	60
	90	12	1,475	70
	100	14	2,500	75

Tables 2 and 3 are reproduced by kind permission of the Department of National Health and Welfare from the *Canadian Bulletin on Nutrition*, 2, 1 (1950), published by the Queen's Printer, Department of Public Printing and Stationery, Ottawa.

APPENDIX III: THREE TYPICAL AFRICAN VILLAGE DIETARIES

Staple diet	Component	g. eaten per head per day	Nutritive value per head per day		
1. Maize	Maize flour	397	Calories	1,779	
	Cassava tuber	25	Proteins	50	g.
	Other roots	8	Fats	16	g.
	Bananas	11	Carbohydrates	359	g.
	Groundnuts	20	Calcium	285	mg.
	Other pulses	20	Iron	25	mg.
	Green leaves	53	Vitamin A	5	i.u.
	Cucurbita	48	Carotene	4·6	mg.
	Other fruit and vegetables	71	Ascorbic acid	85	mg.
	Meat	15	Thiamine	0·8	mg.
	Insects	5	Riboflavin	0·6	mg.
	Maize beer	237	Nicotinamide	9·6	mg.
2. Cassava	Cassava flour	374	Calories	1,695	
	Cassava tuber	48	Proteins	25	g.
	Sweet potato	10	Fats	8	g.
	Groundnuts	7	Carbohydrates	381	g.
	Other pulses	5	Calcium	983	mg.
	Green leaves	22	Iron	21	mg.
	Mangoes	263	Vitamin A	2	i.u.
	Other fruit and vegetables	35	Ascorbic acid	97	mg.
	Fish	61	Carotene	3·4	mg.
	Meat	12	Thiamine	0·6	mg.
	Cassava beer	52	Riboflavin	0·5	mg.
			Nicotinamide	9·4	mg.
3. Rice	Rice	402	Calories	2,167	
	Other grains	46	Proteins	55	g.
	Baobab fruit	46	Fats	42	g.
	Groundnuts	71	Carbohydrates	392	g.
	Other pulses	13	Calcium	258	mg.
	Vegetables	57	Iron	7	mg.
	Fish	12	Vitamin A	8	i.u.
	Meat	10	Carotene	1·0	mg.
	Sugar	3	Thiamine	1·0	mg.
	Oil	2	Riboflavin	0·6	mg.
			Nicotinamide	24·0	mg.
			Ascorbic acid	31	mg.

The evaluation of the nutritive value of these dietaries was done by members of the staff of the Medical Research Council Applied Nutrition Unit; the food composition data are taken from Tables of Representative Values of Tropical Foods, *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 253.

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